# A TOXICITY STUDY ON

# *"SEENA RASA CHENDHURAM"*

**(DISSERTATION SUBJECT)**

*For the partial fulfillment of requirements to the Degree of*

## DOCTOR OF MEDICINE (SIDDHA)


**NATIONAL INSTITUTE OF SIDDHA CHENNAI – 600 047**

**AFFILIATED TO THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY CHENNAI – 600 032**

**DEPARTMENT OF NANJU NOOLUM MARUTHUVA NEETHI NOOLUM**


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# INDEX

# CHAPTER I

## INTRODUCTION

Hundreds of years ago, Siddhars have written elaborately about the Siddha medicines for the welfare of the mankind. Siddhars gain supernatural powers through taking karpam. They dedicated the Siddha science to the service of the humanity. The aim of the Siddha science is the reconstruction of the physical body and reconstruction of the spirit by which the body is being built. siddha science is vast, it emphasized on the wellbeing of physical and mental aspect of the body. To prolong the duration of life and attain salvation, Siddhars briefly explained the rejuvenation processes even long before the Aryan period.

Knowledge of nature is the foundation of Siddha medicine and thus the wonderful cures are achieved by changing the internal causes. The Siddha medicine contains many distinctive qualities such as mental healing, spiritual healing, and pranic healing. Thus the philosophy of curing diseases is quite different from other system of medicine. Siddhars have spoken briefly about the conservation of vital energy & how to utilize it for the development of body and mind of the individual.

For medicine preparations they used the raw materials obtained from mineral, animal or vegetable origin. Siddha system is mainly based on the three humours are known as Vali, Azhal, Iyam. The imbalance between these three humours cause disease. This humoural pathology plays a vital role in Siddha medicine.

Diet also plays an important role in maintaining the three humours in the body. Therefore in the course of Siddha treatment patient should maintain the proper diet advised by the physician to prevent diseases. This was illustrated by an ancient siddhar Thiruvalluvar as,

**மிகினும் குறையினும் நோய்செய்யும் நூலோர்**

**வளிமுதலா எண்ணிய மூன்று.**

. The knowledge of poisons is one of the important branches of Siddha science. Siddhars have unparalleled knowledge in the description of dosage, toxicity and management of poisons. Siddha system also deals with medicines prepared with metals.

In Siddha system of medicine, metals and minerals are widely used to cure the diseases for many years. Siddhars were briefly explained the intoxication process of metals. Thus these metals are purified before the preparation to exclude their toxic effects . It is still more regrettable to note that no research work was taken up in this direction even during the time of Asiatic researches or even subsequently.

Thus ***seena rasa chenduram*** which is prepared from mercury ***(Rasam),*** sulphur***(Gandhagam)*** and alum***( Padikaram)*** is taken as dissertation drug for the evaluation of its toxicity profile. The above ingredients are used to cure chronic, infectious, non infectious ailments in Siddha system of Medicine. As per the text, **"S*eena rasa chendhuram"*** containing *Rasam*(mercury), *Gandhagam*(sulphur) and *Padikaram*(alum) is indicated for moola noi(hemorrhoids) and mega noi(venereal diseases).

(Reference: - *Hakkim P.Muhammad Abdulla Sahib, Anuboga vaithiya navaneetham*, part - 3, page no 21, published by *Thamarai noolagam*, Chennai, year of edition -2005).

Until now there is no scientific evaluation is carried out for its toxicity profile. So there is a need of an hour to evaluate the safety studies on ***"Seena rasa chendhuram"***. In this way, I prefer ***"Seena rasa chendhuram"*** as my dissertation drug and to evaluate the toxicity profile. So, in the present investigation, it is aimed to perform acute and repeated oral toxicity studies. These studies are going to be conducted as per OECD guidelines.

## CHAPTER II

# AIM & OBJECTIVES

**AIM:**

- To evaluate the Safety profile of **"*Seena rasa chendhuram*"** on animal model.

**OBJECTIVE:**

- Acute oral toxicity study of ***Seena rasa chendhuram*** by OECD – 423 Guideline.
- 28 days Repeated oral toxicity study of ***Seena rasa chendhuram*** by OECD – 407Guideline.
- To evaluate the physical and chemical analysis of **"*Seena rasa chendhuram".***

# LITERATURE REVIEW

## இரசம்

**வேறு பெயர்கள் :**

காரம்

சூதம்

புண்ணியம்

கற்பம்

சாமம்

சத்து

சூரியவிரோதி

சாதி

சூத்திரன்

வீரியம்

பாரதம்

கனல்

பூதம்

**ஆதார நூல் :** தசாங்க நிகண்டு.

பூரமாஞ் சூதமென்றும் வேதமென்றும் பேரு

புகழ்மங்கை வாலையென்று மிதற்குப் பேரு

சாரமாம் வாதமென்றும் இதற்குப் பேரு

சத்தமிரசம் என்றும் இதற்குப் பேரு

காரமாம் நீச்சத்தாணி யென்றும் பேரு

கருவானி யென்றதற்குப் பேருண்டாச்சு

சூரமாம் அசுநாதி யென்றும் பேரு

சொல்லிவிட்டோம் பூபதிகேள் ரசத்தின் பேரே

**ஆதார நூல் :** அகத்தியர் ஏமதத்துவம் எனும் பஞ்சகாவிய நிகண்டு.

(பக்க எண்.63)

**சுவை :** அறுசுவை, சிறப்பாய் இனிப்பு.

**வீரியம் :** வெப்ப, சீத வீரியம்.

**செய்கை :** உடல்தேற்றி

உடல் உரமாக்கி

மலம்போக்கி

பித்தநீர் அகற்றி

வீக்க முருக்கி

உமிழ்நீர் பெருக்கி

சிறுநீர் பெருக்கி

மேக நாசினி

**இரசத்தின் நற்குணம் :**

1. குருதியைச் சுத்தி செய்து துர்நீரை நீக்கல்
2. குருதியையும் சுக்கிலத்தையும் பெருக்கல்
3. பசியைத் தூண்டல்
4. கிருமிகளைக் கொன்று புண் புரைகளை ஆற்றல்

5. முக வசீகரத்தை உண்டு பண்ணல்

6. மறதியை ஒழித்து மூளைக்கு கவன சக்தியைத் தரல்

7. நரம்புக் கூட்டங்களை வன்மையுறச் செய்தல்

**தீக்குணம் :**

இரசத்தைச் சரியான முறையில் முடித்து உண்ணாததால் வருங் குற்றங்கள் அநேகம்.

வேகம்

திமிர்

சோம்பல்

தாகம்

போகம்

பாண்டு

பித்தம்

உடல் வெப்பம்

வாய்பிதற்றல்

**இரச சுத்தி :**

1. செங்கழுநீர் சாற்றில் அரைத்தெடுக்க சுத்தியாகும்.

2. தும்பை சமூலச் சாறு விட்டு சூரியபுடம் 10 நாட்களுக்கு வைத்து எடுக்க சுத்தி.

3. வெண்கல சட்டியிலிட்டு மஞ்சள்பொடி போட்டு கலக்கி சீலையில் பிழியவும்.

4. மஞ்சள் பொடியில் 1 வேளை, செங்கல்தூளில் 1 வேளை, இந்துப்புவில் 1 நாள் அரைக்க சுத்தியாகும்.

5. செங்கல் மாத்தூளிலும், மஞ்சள் பொடியிலும் ஒவ்வொரு மணிநேரம் ஆட்டி, சுத்தநீரில் அலம்பி 1 படி மேனிச் சாற்றிலிட்டு அடுப்பேற்றி சாறு சுண்டும்படி எரித்து எடுக்கில் சுத்தியாம்.

**ரசம் சேரும் மருந்துகள் :**

| | | **நூல்** |
|---|---|---|
| 1. | சிவ நாம ரசம் | அனுபோக வைத்திய நவநீதம் 7-ம் பாகம் ப.எண்.76 |
| 2. | சர்வசுர வைரவம் | அனுபோக வைத்திய நவநீதம் 5-ம் பாகம் |
| 3. | பச்சை வெட்டு மாத்திரை | ராமதேவர் எனும் யாகோபு வைத்திய சிந்தாமணி - 700 |
| 4. | வீரரச பதங்கம் | ராமதேவர் எனும் யாகோபு வைத்திய சிந்தாமணி - 700 |
| 5. | கந்தக குழம்பு | கண்ணுசாமி பரம்பரை வைத்திரம் ப.எண்.451 |
| 6. | சப்தரச சிந்தூரம் | சிகிச்சாரத்ன தீபம் ப.எண்.251 |

**இரசம் நஞ்சுக்குறி குணங்கள் :**

தூய்மை செய்யாததும் நன்றாக முடிக்கப் பெறாததும், அளவுக்கு அதிகமானதுமான இரசத்தை உண்பதால் குற்றம் உண்டாகும்.

1. வாயில் அக்கரத்தைப் போல் புண் உண்டாகும்.
2. பற்சந்துகளில் ஈறு கட்டும்.

3. பனங்கள்ளைப் போல் வாய் குழம்பும்.
4. வாய், தொண்டை இவைகள் வெந்து வீங்கும்.
5. குடல், வயிறு இவைகள் புண்ணாகும்.
6. வயிற்றின் மீது பட்டை பட்டையாகத் தேமல் படரும்.
7. ரத்த பேதி, போகங்கெடல் உண்டாதல்.
8. பித்தன் போன்று வாய்பிதற்றும், துணிகளைக் கிழித்து அவிழ்த்தெறியச் செய்யும்
9. கண்பார்வை இழத்தல், காது செவிடுபடல்.
10. செம்படை போல் உடம்பில் படை உண்டாதல்.

**இரச நஞ்சு முறிவு :**

1. துளசி வேர்ப்பட்டைக் குடிநீர்
2. கருவேற் கொப்புளிக் குடிநீர்
3. சுரைக்கருப்பு
4. தயிர் வெல்லம்
5. எருக்குக் கொப்புளிக் குடிநீர்

# கந்தகம்

**வேறு பெயர்கள் :**

காரிழையின் நாதம்

பரைவீரியம்

அதீதப் பிரகாசம்

பீஜம்

செல்விவிந்து

சத்திபீசம்

செந்தூரத்தாதி

தனம்

தேவியுரம்

நாதம்

பொன்வர்ணி

இரச சுரோணிதம்

˙பேரென்ற கேடரியென்றுங் கெவுனமென்றும் பேரு

பேரான பிறானித மென்றும் பேரு

ஆரென்ற சிருவான் வித்தென்றும் பேர்

அசுவி யென்றும் அறு கோகமென்றும் – அதீதப் பேரு

நானென்ற நவகெந்தி யென்றும் பேரு

நற்பாச கெந்தியென்று மதற்குப் பேரு

ஊவென்ற நாதபீச கெந்தி யென்றும்

உரைத்தோம் புலத்தியனே கெந்தியதின் பேரே

ஆதார நூல் : அகத்தியர் ஏமதத்துவம் எனும் பஞ்சகாவிய நிகண்டு

**கந்தகம் – சுத்தி முறைகள் :**

1. பொன்னாங்கண்ணிச் சாற்றைச் சட்டியில் இடவும். கந்தகத்தை அதில் போட்டு சிறுதீயாக எரித்து எடுத்துக் கழுவிக் கொள்ளவும்.

2. குழிவெட்டி சட்டி வைத்து ஆட்டுப்பால் விட்டு, மேல்சட்டியில் கந்தகத்தைப் பரப்பி மூடி எரிக்கச் சுத்தியாகும்.

3. மருதோன்றிக் கற்கத்தைப் பசுவின் தயிரில் கலந்து ஒரு சட்டியிலிட்டுச் சீலையால் வேடு கட்டி, மேல் கந்தகத்தை வைத்து மற்றொரு சட்டியால் மூடிச் சீலை செய்து, குழியில் புதைத்து, மேல் சட்டி மேல் ஐந்து வறட்டி கொண்டு புடமிட, கந்தகம் உருகிக் கீழிறங்கும். இவ்விதம் ஏழுமுறை செய்ய சுத்தியாகும்.

4. வாழைக்கட்டை நீரில் கந்தியைப் பத்துமுறை உருக்கி உருக்கிச் சாய்த்தெடுக்கச் சுத்தியாகும். இம்முறையால், கந்தகத்திலுள்ள எண்ணெய் நீங்கும்.

5. துண்டு துண்டாய் நறுக்கி குழிகட்டி நெல்லில் அவித்து எடுத்துக் கொள்ள சுத்தியாகும்.

**கந்தகம் – சேரும் மருந்துகள் :**

| | | |
|---|---|---|
| 1. | சிந்தாமணிக் குளிகை | அகத்தியர் வைத்திய பிள்ளைத்தமிழ் |
| 2. | கந்தகச் செந்தூரம் | போக முனிவர் 700 |
| 3. | ஏமசண்ட மாருதக் குடோரி | பிரம்மமுனி வைத்திய சூத்திரம் |
| 4. | கந்தக பற்பம் | தேரையர் வைத்தியம் 1000 |

| | | |
|---|---|---|
| 5. | சிப்பித் தைலம் | தேரையர் வைத்தியம் 1000 |
| 6. | சூதகெந்தி தார செந்தூரம் | தேரையர் வைத்தியம் 1000 |
| 7. | சர்வவிட தோடாரிக் குளிகை | போகர் 300 |
| 8. | கந்தக மெழுகு | அகத்தியர் - 600 |
| 9. | கந்தகத் தயிலம் | அகத்தியர் பரிபூரணம் - 400 |
| 10. | கந்தகக் கட்டு | தேரன் வெண்பா |

**கந்தகம் - நஞ்சுக்குறி குணம் :**

- கொடிய நச்சுத்தன்மை உடையது அன்று.
- தூய்மை செய்யும் முறையிலும், செய்முறையும் சரிவரக் கவனம் பெறாத கந்தகத்தை மருந்தாக உட்கொண்டால் நாட்பட்ட காலத்தில் நஞ்சை உண்டாக்கும்.

1. கண்கள் மஞ்சள் நிறமாகப் பூத்திருக்கும்.
2. முகம் வெளுத்திருக்கும்.
3. உடம்பு தன் இயற்கை ஒளி குன்றி பீர்க்கம்பூப் போன்ற நிறமடைந்திருக்கும்.
4. பற்கள் கறுத்துப் பாசியடைந்து விகாரப்பட்டிருக்கும்.
5. இடைவிடாமல் வியர்வை உண்டாகும்.
6. அது மஞ்சள் நிறம் போன்ற சேற்று நீரைப் போன்றிருக்கும்.
7. சிறுதீர் வெள்ளாட்டு நீரைப் போன்றிருக்கும்.
8. மலம் சாமந்திப்பூவைப் போன்று மஞ்சள் நிறமாகயிருக்கும்.

9. வாயில் புகை நாற்றமுண்டாகும்.

10. பொய்ப்பசி, வயிற்றுவலி, வயிற்றுப்பிசம், வியர்க்குரு போன்ற பல குணங்களை உண்டாக்கும்.

**கந்தகம் – நஞ்சு முறிவு :**

1. ஆவாரம்வேர், தைவேளை வேர், நீலிவேர், சுக்கு, பருத்தியிலை, சிறுநாகப்பூ இவற்றை குடிநீரிட்டுக் கொடுக்க கந்தகக் குற்றம் நீங்கும்.

2. தாமரை வித்தை இளநீரில் அரைத்துண்ணத் தீரும்.

3. மிளகு, நீலிவேர், சீரகம் சரியெடை எடுத்துக் குடிநீரிட்டுக் கொடுக்க கந்தக நஞ்சு முறியும்.

# படிகாரம்

**வேறு பெயர் :**

படிகாரம்

படிகி

சீனம்

சாற்றீனோஞ் சிவமூல காரமென்றும் பேரு

சதாசிவ சத்திவரிநார மென்றும் பேரு

போற்றினோம் பூபதி காரமென்றும் பேரு

பூட்டினோம் பூவாதி காரமென்றும் பேரு

வேற்றினோம் வேதியன் காரமென்றும் பேரு

விலாட யுரகார மென்றுமதற்குப் பேரு

வாழ்த்தினோம் வாலை ரகுபதி காரமென்றும்

வளமாக வசனித்தோஞ் சீனாக்காரத்தின் பெயரே

ஆதார நூல் : அகத்தியர் ஏமதத்துவம் எனும் பஞ்சகாவிய நிகண்டு

**சுத்திமுறைகள் :**

1. நீரில் கரைத்து வடிகட்டிக் காய்ச்சிக் குழம்பு பக்குவத்தில் இறக்கிக் குளிரும்படி செய்ய சுத்தியாம்.
2. பசும்பாலில் 9 மணி நேரம் ஊறவைத்து எடுக்கச் சுத்தியாகும்.
3. சிறுநீரில் மூன்று நாட்கள் ஊறவைத்து எடுத்துக் கொள்ள சுத்தியாகும்.
4. படிகாரத்தை எருமைச் சாணத்தில் கிழிகட்டி வைத்துக் கொள்ள சுத்தியாகும்.

**சேரும் மருந்துகள் :**

| | | |
|---|---|---|
| 1. | படிகார மாத்திரை | கண்ணுசாமிப் பரம்பரை வைத்தியம் |
| 2. | சீனப்புகை | அகத்தியர் வைத்திய காவியம் 1500 |
| 3. | பச்சைக்குழம்பு | அகத்தியர் வைத்திய பிள்ளைத்தமிழ் |
| 4. | அறுவகைக் குழம்பு | பிரம்மமுனி வைத்திய சூத்திரம் |

**படிகாரம் – உபயோகங்கள் :**

1. தீராத வாந்திக்கு கடுக்காய், சுக்கு, வெங்காரம், சீனாக்காரம், இவைகளுடன் சர்க்கரையும், தேனும் கூட்டி எலுமிச்சம்பழச்சாற்றால் அரைத்து நாக்கில் தேய்த்துவிட நிக்காத வாந்தியும் நிற்கும்.

2. இரண்டு உளுந்தெடை படிகாரத்தை ஓர் அவுன்ஸ் நீரில் கரைத்து கண் கழுவ கண்ணோய் நீங்கும்.

3. ஒரு பலம் படிகாரத்தை எட்டு படி நீரில் கரைத்து, அந்நீரை அக்கரத்தில் வாய் கொப்புளிப்பதற்கும், புண்களைக் கழுவுவதற்கும் உபயோகிப்பதுண்டு.

4. வெட்டுப்பட்ட இடங்களில் படிகாரத்தை நீரில் கரைத்து சீலையில் நனைத்துக் கட்ட அது குருதிப்பெருக்கை அடக்கும்.

5. நீரில் கரைத்து மூக்கு, குய்யம், எயிற்றடி இவ்விடங்களில் வரும் ரத்தத்தை நிறுத்தத் தடவலாம்.

# MERCURY

## OVERVIEW:

Mercury is a heavy metallic element with the unique property of being liquid at standard atmospheric pressure and temperature. The term `heavy metal' should probably be reserved for those elements with an atomic mass of 200 or greater [e.g., mercury (200), thallium (204), lead (207), bismuth (209) and the thorium series]. Mercury has been known for thousands of years. In many cultures, people learned to make mercury metal from its most important ore, cinnabar. When heated cinnabar releases mercury as a vapor (gas). The vapor is cooled and captured as liquid mercury.

Mercury is a transition metal. A transition metal is one of the elements found between Groups 2 (IIA) and 13 (IIIA) on the periodic table. Mercury has long been known as quicksilver, because it is a silver liquid. The chemical symbol also reflects this property. The symbol Hg comes from the Latin term *hydrargyrum,* meaning "watery silver".

## IDENTIFICATION DATA:

| | |
|---|---|
| Symbol | : Hg |
| Atomic Number | : 80 |
| Atomic Mass | : 200.59 |
| Family | : Group 12 (IIB)<br>Transition metal |
| **CAS Number** | **: 7439-97-6** |
| ECNumber(EINECS) | : 231-106-7 |
| Synonyms | : Hg, Colloidal mercury, Kwik, Mercure, Mercurio, Metallic mercury, NA 2809, NCI-C60399, Quecksilber, Quick silver, Rcra waste number U151, Rtec, UN 2809, Blue mass, Hydrargyrum, Liquid silver, Mercury mass |
| Appearance | : Silvery liquid metal |

**SOURCE:**

**Occurrence in nature:**

The abundance of mercury in the Earth's crust is estimated to be about 0.5 parts per million. That makes it one of the 20 least common elements. It very rarely occurs as an element. Instead, it is usually found as a compound. Its most common ore is cinnabar, or mercuric sulfide (HgS). Cinnabar usually occurs as a dark red powder. It is often called by the common name of vermillion or Chinese vermillion. The largest producer of mercury outside the United States is Spain. U.S. production numbers are not announced in order to protect U.S. industries from revealing important company secrets. Other producers after Spain are Kyrgyzstan, Algeria, China, and Finland. In the United States, mercury is produced as a by-product of gold mining. It comes from eight gold mines in California, Nevada, and Utah.

**Discovery and naming:**

The oldest sample of mercury dates to about the fifteenth or sixteen century B.C. It was found in an Egyptian tomb at Kurna, stored in a small glass container. Mercury and cinnabar are both mentioned in ancient manuscripts. The Chinese, Hindus, Egyptians, Greeks, and Romans all recorded information about the element and its ore. Greek philosopher Theophrastus (372-287 B.C. ), for example, described a method for preparing mercury. Cinnabar was rubbed together with vinegar in a clay dish. Theophrastus wrote that the cinnabar had been found in **silver** mines. When the metal was first made, he said, people thought it might contain **gold.**They were misled by the metal's shiny appearance. They soon realized, however, that it was quite different from gold. In the last forty years, the dangers of mercury have become better known. As a result, mercury use is now being phased out. Many reports on mercury told of its poisonous effects. Slaves who worked in Roman mercury mines, for example, often died of exposure to mercury. Strangely enough, trees and plants around these mines were not affected. Mercury was sometimes very dangerous and sometimes quite safe. People even drank from streams that ran through mercury mines. Scientists now know that mercury's effects depend on the form in which it occurs. Mercury amalgams have also been around for a long time.

An amalgam is a combination of mercury with at least one other metal. Amalgams are formed when a metal, such as silver, dissolves in mercury. The process is

similar to dissolving salt in water. Amalgamation is used in mining to remove silver from ore. The silver dissolves in the mercury and a silver amalgam is formed. Heating the amalgam releases the silver. This method was used by miners as early as the sixteenth century.

**Physical properties:**

Mercury is the only liquid metal. In fact, there is only one other liquid °C (–37.93°F). It can be element,**bromine.** Bromine is a non-metal. Mercury can be frozen (changed into a solid) at a temperature of –38.85changed into a gas ("boiled") at 365.6°C (690.1°F). Its density is 13.59 grams per cubic centimeter. Mercury has two physical properties of special interest. First, it has very high surface tension. Mercury is also a very good conductor of electricity.

**Cleavage** **:** None

**Color** **:** Tin white, Gray white.

**Density** **:** 13.6

**Diaphaneity** **:** Opaque

**Habit** **:** Liquid - Occurs as a liquid at room temperatures.

**Hardness** **:** 0 - Liquid

**Luminescence** **:** Non-fluorescent.

**Luster** **:** Metallic

**Magnetism** **:** Nonmagnetic

**Streak** **:** none

**Melting Point** **: -39$^{o}$ C**

**Boiling Point** **: 357$^{o}$ C**

**Vapor Pressure** **: 0.002 mm Hg at 20$^{o}$ C**

**Chemical properties:**

Mercury is moderately active. It does not react with **oxygen** in the air very readily. It reacts with some acids when they are hot, but not with most cold acids.

**Occurrence in nature:**

The abundance of mercury in the Earth's crust is estimated to be about 0.5 parts per million. That makes it one of the 20 least common elements. It very rarely occurs as an element. Instead, it is usually found as a compound. Its most common ore is cinnabar, or mercuric sulfide (HgS). Cinnabar usually occurs as a dark red powder. It is often called by the common name of vermillion or Chinese vermillion. The largest producer of mercury outside the United States is Spain. U.S. production numbers are not announced in order to protect U.S. industries from revealing important company secrets. Other producers after Spain are Kyrgyzstan, Algeria, China, and Finland. In the United States, mercury is produced as a by-product of gold mining. It comes from eight gold mines in California, Nevada, and Utah.

**Isotopes:**

Seven naturally occurring isotopes of mercury are known. They are mercury-196, mercury-198, mercury-199, mercury-200, mercury-201, mercury-202, and mercury-204. Isotopes are two or more forms of an element. Isotopes differ from each other according to their mass number. The number written to the right of the element's name is the mass number. The mass number represents the number of protons plus neutrons in the nucleus of an atom of the element. The number of protons determines the element, but the number of neutrons in the atom of any one element can vary. Each variation is an isotope.

Mercury is the only liquid metal. About a dozen radioactive isotopes of mercury are known also. A radioactive isotope is one that breaks apart and gives off some form of radiation. Radioactive isotopes are produced when very small particles are fired at atoms. These particles stick in the atoms and make them radioactive. Two radioactive isotopes of mercury are used in medicine, mercury-197 and mercury-203. Both isotopes are used to study the brain and the kidneys. The isotopes are injected into the body where they travel to the brain and the kidneys. Inside these two organs, the isotopes give off radiation that is detected by instruments held above the body. The pattern of radiation provides information about how well the brain and kidneys are functioning.

**Extraction:**

Mercury is still prepared as it was hundreds of years ago. Cinnabar is heated in air. The compound breaks down to give mercury metal:

**Purity:**

The mercury metal is then purified by distillation. Distillation is the process of heating two or more liquids to their boiling points. Different liquids boil at different temperatures. The liquid that is wanted (such as mercury) can be collected at *its* boiling point. Mercury that is more than 99 percent pure can be collected by distillation.

**Uses:**

The most important use of mercury is in the preparation of chlorine. Chlorine is produced by passing an electric current through sodium chloride. Notice that two different endings are used for mercury compounds. Those that end in *-ous* have less mercury than those that end in *-ic*.

Mercuric arsenate ($HgHAsO_4$) : Waterproofing paints

Mercuric benzoate ($Hg(C_7G_5O_{2)2}$): Medicine, used to treat syphilis

Mercuric chloride, or mercury dichloride, or corrosive sublimate ($HgCl_2$): disinfectant, tanning of leather, spray for potato seedlings (to protect from disease), insecticide, preservation of wood, embalming fluid, textile printing, and engraving

Mercuric cyanide ($Hg(CN)_2$): germicidal soaps (soaps that kill germs), photography

Mercuric oxide (HgO): red or yellow pigment in paints, disinfectant, fungicide (to kill fungi), perfumes and cosmetics

Mercuric sulfide (HgS): red or black pigment in paints

Mercurous chloride, or calomel ($Hg_2Cl_2$): fungicide, maggot control in agriculture, fireworks

mercurous chromate ($Hg_2CrO_4$): green pigment in paints

Mercurous iodide ($Hg_2I_2$): kills bacteria on the skin

Mercury was also widely used in dentistry. It was used to make amalgams, alloys of mercury with other metals, used to fill teeth. Most people even today are likely to have dental fillings that contain a small amount of mercury metal.

**Acute and chronic toxic effects of mercury:**

**Inorganic mercury exposure:**

Exposure to inorganic mercury or mercuric salt occurs mainly through the oral and GI tract. Its corrosive properties account for most of the acute signs and symptoms of inorganic mercury or mercuric salt toxicity. The acute presentation can include ashen-gray mucous membranes secondary to precipitation of mercuric salts vomiting, severe abdominal pain, and hypovolemic shock.

Systemic effects usually begin several hours postingestion and may last several days. These effects include metallic taste, stomatitis, gingival irritation, foul breath, loosening of teeth and renal tubular necrosis leading to oliguria or anuria.

Some unique features of chronic mercurial poisoning (hydragyrism):

- Metal fume fever - Occurs in the acute phase of mercury vapor toxicity and is manifested by fatigue, weakness, fever, chills, dizziness, headache, abdominal cramping, dyspnea, dysuria, and ejaculatory pain.
- Acrodynia- Also known as pink disease and considered to be a mercury allergy; presents with erythema of the palms and soles, edema of the hands and feet, desquamating rash, hair loss, pruritus, diaphoresis, tachycardia, hypertension, photophobia, irritability, anorexia, insomnia, poor muscle tone, and constipation or diarrhea; acrodynia does not present in everyone who is exposed to inorganic mercury, but it is an indicator of widespread disease.
- Erethism - A constellation of irritability, excitability, anxiety, insomnia, and social withdrawal; erethism traditionally is seen in the chronic phase of the toxicity.
- Danbury tremors - occur first in the hands,then progress to lips and tongue and finally involve arms and legs.the tremor is moderately coarse and is interspersed by jerky movements.
- Hatter's shakes or glass-blower's shakes – these are common in person's working in glass-blowing and hat industries.the patient then become unable to dress himself.
- Concussio mercurialis – in this condition no activity is possible.

Chronic and intense, acute mercury exposure cause cutaneous and neurologic symptoms. The classic triad found in chronic toxicity is tremors, gingivitis, and erethism. Additional findings may include headache, visual disturbance (eg, tunnel vision), peripheral neuropathy, salivation, insomnia, and ataxia.

**Organic mercury exposure:**

Organic mercury poisoning usually results from ingestion of contaminated food. The long-chain and aryl forms of organic mercury have similar toxic characteristics as inorganic mercury. The onset of symptoms usually is delayed (days to weeks) after exposure. Organic mercury targets enzymes, and the depletion of these enzymes must occur before the onset of symptoms.

# SULFUR

**Introduction:**

Sulfur is a mineral which exists as a natural production and occurs abundantly and uncombined in nature. It is found particularly in the neighborhood of volcanoes such as in Sicily, Japan, Iceland, Sumathra, philipine and Island's etc. In India it occurs naturally in some parts like Nepal and Cashmere. Sulfur occurs in combination with many metals such as Copper, Iron etc. Few vegetables and some animal products contain sulfur in small quantities.

**History:**

Sulfur has been known since prehistoric times. Because it is flammable, alchemists regarded sulfur as essential to **combustion.** The chemical properties of sulfur and its compounds, including the reaction of sulfur with mercury (Hg) to form a red solid, mercuric sulfide (HgS), and the use of sulfuric acid ($H_2SO_4$) as solvent of **metals**, were discovered at about C.E. 250–300. Gunpowder, a mixture of sulfur, charcoal, and potassium nitrate ($KNO_3$), was first used for military purposes in China in C.E. 904.

**Availability:**

Sulfur is procurable in bazaars in different forms as mentioned below but among those, the myrobalan sulfur which is the purest of all the one used in medicine. Form sulfur are prepared sublimated sulfur, amorphous sulfur, green vitriol, white vitriol, blue stone, oxides of mercury etc. Myrobalan gandac or rhombic sulfur is the purest of the all kinds and is of bright yellow color with a shade of green . It is procurable in all bazaars in crystalline pieces. It is this which is much used in medicine and is so called from its resemblance to Indian gooseberry in color.

**Physico - chemical Properties:**

Sulfur is a tasteless, odorless, nonmetallic element. Sulfur along with selenium (Se) and tellurium (Te) are called chalcogens. The valences of sulfur are 2, 4, and 6, which can be represented by compounds such as hydrogen sulfide ($H_2S$), sulfur dioxide ($SO_2$), and barium sulfate ($BaSO_4$), respectively. Pure sulfur is insoluble in water. The most stable variety of sulfur, rhombic sulfur, is a yellow crystalline solid.

Color : Yellow, Yellowish brown, Yellowish gray, Reddish, Greenish.

Density : 2.05 - 2.09, Average = 2.06

| | |
|---|---|
| Diaphaneity | : Transparent to translucent |
| Fracture | : Sectile - Curved shavings or scrapings produced by a knife blade, (e.g.graphite). |
| Habit | : Massive - Uniformly indistinguishable crystals forming large masses. |
| Hardness | : 1.5-2.5 - Hardness very near Gypsum |
| Luminescence | : Non-fluorescent. |
| Luster | : Resinous |
| Streak | : white |
| Chemical Name | : Sulfur |
| Chemical Formula | : S |
| **CAS Number** | **: 7704-34-9** |
| EC Number (EINECS): | 231-722-6 |
| Synonyms | : Sulfur, Precipitated, S, Brimstone, Colloidal sulfur, Flowers of sulphur, Precipitated sulfur, component of Bensulfoid, Asulfa-Supra, Atomic sulfur, Bensulfoid, Colloidal-S, Colsul, Corosul D and S, Cosan, Cosan 80, Crystex, Elosal, Flour sulfur, Ground vocle sulfur, Hexasul, Kolloidschwefel 95, Kolo 100, Kolofog, Kolospray, Kumulus, Magnetic 70, 90, and 95, Micowetsulf, Microflotox, Netzschwefel, Polsulkol Extra, RC-Schwefel Extra, Sofril, Sperlox-S, Sp |
| Molecular Weight | : 32.066 |

**PURITY:**

Pure sulfur exists in two stable crystalline forms (a) Alpha-sulfur: rhombic, octahedral yellow crystals stable at room temperature. (b) Beta-sulfur: monoclinic, prismatic pale yellow crystals slowly changing to alpha form below 94.5°C. Both forms are insoluble in water, but slightly soluble in alcohol and ether. Soluble in carbon disulfide, carbon tetracholoride, benzene, Conbustible, nontoxic. Rhombic sulfur is made up of eight sulfur atoms that are linked in the form of a ring, which is rhombic when the sulfur is at room temperature--hence its name. A simple test can be used to determine the purity of a rhombic sulfur sample. The principle premise of the test is pretty straight forward, in that it makes use of weight differences to determine the percentage ash content in a given sample. The following parameters are most frequently measured for quality control purposes.

· Moisture Content

· Ash Content

· Acidity

· Chloride Content

· Carbon Content (Organics)

· Hydrogen Sulphide Content

· Ammonia Content

· Arsenic

· Iron

**General uses of sulfur:**

Sulfur is of 4 different colours viz; red, yellow, white and blue or black, of which the red variety is used in alchemy, the yellow in medicine, the white and blue or black for rejuvenation, but the lost mentioned are said to be obtained very rarely and with much difficulty.

Sulfur finds extensive technological applications such as in production of sulfuric acid, plastics, enamels, antimicrobial agent, insecticide, fumigant, metal glass cements, in manufacture of dyes, phosphate fertilizers, gun-powder and in the vulcanization of rubber, etc. [1-4]. Sulfur nanostructures are also used in synthesis of sulfur nanocomposites for lithium batteries [5,6], modification of carbon nanostructures [7,8], in synthesis of sulfur nanowires with carbon to form hybrid materials with useful properties for gas sensor and catalytic applications [9], Metal-sulfur compounds like ZnS and CdS play important role in nonlinear optical and electroluminescent devices, etc. [10-15].

Sulfur is commercially important in the manufacture of chemicals such as sulfuric acid. The chemicals, in turn, are used in the manufacture of sulfa drugs, **vulcanized rubber**, acid batteries, dyes, and so on. In agriculture, sulfur is the fourth most important crop nutritive element, after nitrogen, phosphorus, and potassium. Its use in fertilizers is increasing rapidly. Sulfur is also used to manufacture poultry feed additives, pesticides, and parasiticides.

Sulfur is used in medicine only after it is refined and cleaned according to the rules laid down in Tamil medical science because impure sulfur is said to bring on diseases or other complications.

**Therapeutic uses of sulfur:**

- Sulfur is chiefly used in scabies and skin diseases both as an external and internal remedies .
- The fumes of burning sulfur are said to cure gout and rheumatic affection.
- When mixed with coconut oil, it is applied to itches and sores.
- When mixed with neem oil it is applied to rheumatic affection.
- When mixed with gingili oil and powdered black cumin seed, its is given internally for venereal herpes.
- The calcined sulfur (Contemplated in Tamil medicine) is prescribed by vaidyars for leprosy (Lepra arabum), Jaundice, Contracted limbs, venereal diseases etc.
- The acid prepared from sulfur is corrosive and will raise blisters on the body, when coming in contact with it.
- Inorganic sulfur reduces the motility and invasion of MDA-MB-231 human breast cancer cells.

**Reactions:**

Sulfur dioxide is the product of the burning of materials that contain sulfur:

$$S_8 + 8\ O_2 \rightarrow 8\ SO_2$$

Sulfur reacts with sulfite ions in solution to form thiosulfate:

$$S + SO_3^{2-} = S_2O_3^{2-},$$

but the reaction is reversed in an acidic solution.

**Sulfuric Acid:**

Sulfuric acid is produced by the contact process in three steps:

$S+O_2 +H_2SO_4+H_2O$

$SO_2$ --> $SO_3$ -----> $H_2S_2O_7$ ---> $H_2SO_4$

**Toxicological aspects:**

**Sulfur toxicity** is associated mainly with high levels of the element and its toxic volatile substances in the environment. **Sulfur** dioxide (SO(2)), a major air pollutant, may adversely affect animal and human health by causing bronchitis, bronchoconstriction, and increased pulmonary resistance. Sulfur dioxide is toxic in large amounts. It or its conjugate base bisulfite is produced biologically as an intermediate in both sulfate-reducing organisms and in sulfur oxidizing bacteria as well. Being easily condensed and possessing a high heat of evaporation, sulfur dioxide is a candidate material for refrigerants. Prior to the development of CFCs, sulfur dioxide was used as a refrigerant in home refrigerators. Sulfur, usually in the form of hydrogen sulfide (H2S), and to a much lesser extent, sulfur dioxide (SO2), methyl mercaptan (CH4S), carbonyl sulfide (OCS), and carbon disulfide(CS2).

All of these sulfur compounds are corrosive, toxic, and are serious atmospheric pollutants. Hydrogen sulfide will attack most common metals, and also many plastics. The cost of removing sulfur from low quality "sour gas" can easily outweigh the potential profits.

# ALUM

**Introduction:**

Potassium alum, which is the hydrated form of potassium aluminum sulfate and has the chemical formula $KAl(SO_4)_2 \cdot 12H_2O$. However, any of the compounds with the empirical formula $AB(SO_4)_2 \cdot 12H_2O$ are considered to be alum. Sometimes alum is seen in its crystalline form, although it is most often sold as a powder. Potassium alum is a fine white powder that you can find sold with kitchen spices or pickling ingredients. It is also sold as a large crystal as a "deodorant rock" for underarm use.

**Types of Alum:**

- **Potassium Alum**

  Potassium alum is also known as potash alum or tawas. It is aluminum potassium sulfate. This is the type of alum that you find in the grocery store for pickling and in baking powder. It is also used in leather tanning, as a flocculant in water purification, as an ingredient in aftershave and as a treatment to fireproof textiles. It's chemical formula is $KAl(SO_4)_2$.

- **Soda Alum**

  Soda alum has the formula $NaAl(SO_4)_2 \cdot 12H_2O$. It is used in baking powder and as an acidulent in food.

- **Ammonium Alum**

  Ammonium alum has the formula $NH_4Al(SO_4)_2 \cdot 12H_2O$. Ammonium alum is used for many of the same purposes as potassium alum and soda alum. Ammonium alum finds applications in tanning, dyeing textiles, making textiles flame retardant, in the manufacture of porcelain cements and vegetable glues, in water purification and in some deodorants.

- **Chrome Alum**

  Chrome alum or chromium alum has the formula $KCr(SO_4)_2 \cdot 12H_2O$. This deep violet compound is used in tanning and can be added to other alum to grow lavender or purple crystals.

- **Selenate Alums**

  Selenate alums occur when selenium takes the place of sulfur, so that instead of a sulfate you get a selenate, ($SeO_4^{2-}$). The selenium-containing alums are strong oxidizing agents, so they can be used as antiseptics, among other uses.

- **Aluminum Sulfate**

  This compound is also known as papermaker's alum. However, it is not technically an alum.

**General properties**

| | |
|---|---|
| Appearance | : silver foil, shot or powder |
| Stability | : Stable. Powder is flammable. Reacts very exothermically with halogens. |
| Alternate Names | : Aluminum AL |
| Symbol | : Al |
| Atomic Number | : 13 |
| Atomic Weight | : 26.98154 |
| Melting Point | : 660.37$^{o}$C |
| Boiling Point | : 2467 $^{o}$C |
| Thermal Conductivity | : 2.37 W/cm/ K @ 298.2 K |
| Electrical Resistivity | : 2.6548 microhm-cm @ 0 $^{o}$C |
| Electro negativity | : 1.5 Paulings |
| Specific Heat | : 0.215 Cal/g/ K @ 25 $^{o}$C |
| Heat of Vaporization | : 67.9 K-Cal/gm at om at 765 $^{o}$C |
| Heat of Fusion | : 2.55 Cal/gm mole |

**Physico chemical properties:**

| | |
|---|---|
| Cleavage | : {111} Indistinct |
| Color | : Colorless, White. |
| Density | : 1.76 |
| Diaphaneity | : Transparent |
| Fracture | : Conchoidal - Fractures developed in brittle materials Characterized by smoothly curving surfaces (e.g. quartz). |
| Habit | : Water Soluble - Water soluble mineral. |
| Hardness | : 2 - Gypsum |
| Luminescence | : Non-fluorescent. |
| Luster | : Vitreous (Glassy) |
| Streak | : white |
| Chemical Formula | : KAl(SO4)2•12(H2O) |
| **CAS Number** | **: 7429-90-5** |
| **EC Number (EINECS)** | : 231-072-3 |
| **Synonyms** | **:** Al, Aluminium powder, A 00, A 95, A 99, A 995, A999, AA 1099, AA1199, AD 1, AD1M, ADO, AE, Alaun, Allbri aluminum paste and powder, Alumina fibre, Aluminium, Aluminium bronze, Aluminium flake, Aluminum 27, Aluminum A00, Aluminum dehydrated, Aluminum metal, Aluminum pyro powders, AO A1, AR2, AV00, AV000, C.I. 77000, Emanay atomized aluminum powder, JISC 3108, JISC 3110, L16, Metana, Metana aluminum paste, PAP-1, UN 1309, UN 1383, UN 1396. |
| **Appearance** | **:** Silver foil, shot or powder |
| **Stability** | **:** Stable. Powder is flammable. Reacts very Exothermically with halogens. Moisture and air sensitive. Incompatible with strong acids, caustics, strong oxidizing agents, halogenated hydrocarbons. |
| **Alternate Names** | **:** Aluminum \| AL |

**REACTIONS**

The balanced chemical equation for this oxidation-reduction reaction is

$$2\ Al\ (s) + 2\ KOH\ (aq) + 6\ H_2O\ (l) \longrightarrow 2\ KAl(OH)_4\ (aq) + 3\ H_2\ (g)$$

The second step of the procedure is to convert the $KAl(OH)_4$ to alum by addition of sulfuric acid ($H_2SO_4$) in an acid-base reaction. Under the experimental conditions, the alum has a limited solubility in water, and so it precipitates from the solution. The balanced chemical reaction that occurs in this step is

$$KAl(OH)_4\ (aq) + 2\ H_2SO_4\ (aq) + 8\ H_2O\ (l) \longrightarrow KAl(SO_4)_2 \cdot 12\ H_2O\ (s)$$

The overall balanced chemical reaction for the conversion of aluminum to alum, shown below, can be obtained by adding together the balanced chemical equation for each step (Help Me).

$$2\ Al\ (s) + 2\ KOH\ (aq) + 22\ H_2O\ (l) + 4\ H_2SO_4\ (aq) \longrightarrow 2\ KAl(SO_4)_2 \cdot 12\ H_2O\ (s) + 3\ H_2\ (g)$$

Alum is an ionic compound, which means its melting and boiling points are likely to be too high to be measured convenient. When placed in a flame, aluminum does not change the flame's color, and so a visual flame test cannot be used to show the presence of Al. Alum is a hydrate, which means that it is a compound that has water molecules trapped within the solid. Hydrates will release some, or all, of their "waters of hydration" upon heating.

**Source**

It is found native in Italy, in the neighborhood of volcanoes, and is the mineral from which the metal aluminum is obtained.

**Derivation**

Alum is also obtained from aluminous slate, shale or schist, by the process of roasting and exposure to the air. Alum is a white, slightly efflorescent salt, which crystallizes in regular octahedrons. It possesses an astringent, acid, and sweetish taste. It is insoluble in alcohol, but dissolves in from fourteen to fifteen times its weight in cold, and three-fourths of its weight in boiling water.

**Uses of Alum**

Alum has several household and industrial uses. Potassium alum is used most often, although ammonium alum, ferric alum and soda alum may be used for many of the same purposes.

- purification of drinking water as a chemical flocculant
- in styptic pencil to stop bleeding from minor cuts
- adjuvant in vaccines (chemical that enhances immune response)
- deodorant "rock"
- pickling agent to help keep pickles crisp
- flame retardant
- the acidic component of some types of baking powder
- an ingredient in some homemade and commercial modeling clay
- an ingredient in some depilatory (hair removal) waxes
- skin whitener
- ingredient in some brands of toothpaste

**Medical Properties and Action:**

Alum is astringent and styptic, and is employed both externally and internally. When taken internally, it is absorbed into the system, and has been detected in the liver, spleen and urine. Excessive doses cause vomiting, griping, purging, and inflammation of the gastro-enteric mucous membrane. Powdered alum, in doses of a teaspoonful, is an efficient emetic. It coagulates albumen and causes an abundant flow of saliva, coagulating the albumen of the saliva and buccal mucus in whitish, membranous flakes. Its astringent influence is chiefly upon mucous surfaces. Applied locally to relax or bleeding parts, it corrugates the surrounding tissues and causes contraction of the capillaries, and, in this manner, acts as an astringent.

**Therapeutic Uses**

Alum is internally administered in diarrhoea, chronic dysentery, colica pictonum, catarrh of the stomach, etc. Externally it is applied in ulcerated and relaxed throat affections, ptyalism, gonorrhoea and gleet, uterine hemorrhage, morbid growths, hematuria, ophthalmia, chronic whooping-cough, chronic skin diseases, chilblains, ulcers, hospital gangrene, etc., etc.

# CHAPTER IV

## MATERIALS AND METHODS

### 4.1 COLLECTION, IDENTIFICATION, PURIFICATION AND DRUG PREPERATION:

**Test drug: *Seena rasa chendhuram***

***Seena rasa chendhuram*** contains the following incredients :

| | | |
|---|---|---|
| Purified *Padikaram* (alum) | – | 1 *palam*(35gm). |
| Purified *Gandhagam* (sulphur) | _ | 3 *palam*(105gm). |
| Purified *Rasam*(mercury) | _ | 2 *palam*(70gm). |

**Collection**

The raw drugs are obtained from the standard raw drug markets in Chennai, Tamilnadu.

**Authentication**

Drugs were identified and authenticated from dept. of Pharmacognosy in Siddha Central Research Institute, Chennai.

**Method of Purification**

**Purification of *Padikaram*(alum)**

Alum is tide up in a piece of cloth and suspended in a solution of buffalodung. Then alum is taken off from the buffalodung and washed well.

***- Sarakku sutthi sei muraigal***

**Page No:86**

**Purification of *Gandhagam* (sulphur)**

Raw sulphur is boiled in goat's milk, washed and then dried.

***- Sarakku sutthi sei muraigal***

**Page No.65**

**Purification of *Rasam*(mercury)**

35gm of rasam is soaked in leucas aspera juice for one day and kept in sunlight. The process is repeated for 10 days by adding fresh leucas aspera juice daily. Then the mixture is dried in sunlight for another 10 days until it is dried completely.

**Method of Preparation of *seena rasa chendhuram***

Purified *Rasam,* Purified *Gandhagam* and Purified *Padikaram* are packed in a container, buried in husk and baked in *pudam* . Then the baked drug is powdered and taken.

Dose of drug : One – Two *kundri* (130 – 260mg)

Adjuvant : Ghee / Butter

Duration : 5 days

Therapeutic uses : *Moola noi*, Moola vayu, Mega noi and Kasam.

***- Anupoga vaithiya navaneetham part III***

**Page No: 21**

**Edition - 2005**

**Fig .1.UN PURIFIED MERCURY**

**Fig .2. PURIFIED MERCURY**

**Fig .3.UN PURIFIED GANDHAGAM**

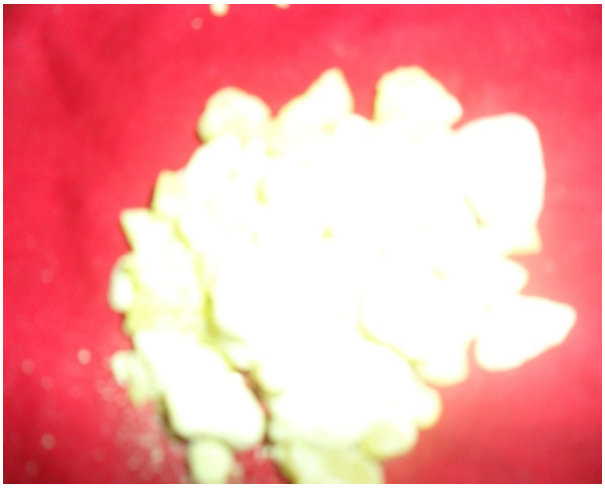


**Fig .4. PURIFIED GANDHAGAM**

**Fig .5. UNPURIFIED PADIKARAM**

**Fig .6. PURIFIED PADIKARAM**

**Fig .7. SEENA RASA CHENDHURAM**

## 4.2 QUALITATIVE ANALYSIS

### PHYSICO CHEMICAL PROPERTIES

**Sample discription:** ***seena rasa chendhuram***

### COLOUR

About 50 gm of ***seena rasa chendhuram*** was taken in a clean glass beaker and tested for its colour by viewing again a white opaque back ground under direct sunlight.

### ODOUR

About 50 gm of the ***seena rasa chendhuram*** was placed in 100 ml of beaker and tested for its odour by wafting the air above the beaker.

### LOSS ON DRYING@100 ºC

5 gms of ***seena rasa chendhuram*** was heated in a hot oven at 100º c to a constant .The percentage of loss of weight was calculated.

### pH

The pH of the ***seena rasa chendhuram*** was estimated as per the method prescribed in the Indian standard (IS) -6940(1982). One gram of the ***seena rasa chendhuram*** was taken in to a 100ml graduated cylinder containing about 50 ml of water and filled up to the mark with water. The cylinder was stopped and shaken vigorously for two minutes and the suspension was allowed to settle for hour at 25ºc to 27ºC about 25 ml of the clear aqueous solution was transfered in to a 50 ml beaker and tested for pH using DIGISUN digital pH meter (DIGISUN electronics, Hyderabad, India)

### DETERMINATION OF ASH VALUE

Weighed accurately 2gms of the ***seena rasa chendhuram*** in tarred platinum or silica dish and incinerate at a temperature not be exceeding 450ºC until free from corban cooled and weighed calculate the percentage of ash with reference of the air dried drug was then calculated.

**WATER SOLUBLE ASH**

To the Gooch curable containing to the total ash, added 25 ml of water and boiled for 5 minutes. Collected the insoluble matter in a sintered glass crucible for 15 minutes at a temperature not exceeding 450º c subtract the weight off the insoluble matter from the weight of the ash the difference of the weight represents the water soluble ash. Calculate the percentage of water soluble ash with the reference to the air dried drug.

**ACID INSOLUBLE ASH**

Boiled ash 5minutes with 25 ml of 1:1 dil. Hcl collect the insoluble matter Gooch crucible on an ash less filter paper wash without water and ignited, cooled in a desiccators and weighed , calculated the percentage of insoluble ash with reference to the air dried drug.

**LOSS ON DRYING**

Five grams of the S***eena rasa chendhuram*** is heated in a hot oven at 105ºC to constantly, and weighed. The percentage of loss of weight was calculated there from.

# QUALITATIVE ANALYSIS

## CHEMICAL ANALYSIS

**Sample discription:** ***seena rasa chendhuram***

| **EXPERIMENT** | **OBSERVATION** | | | | | **INFERENCE** |
|---|---|---|---|---|---|---|
| | ***Gandhagam* B.F** | ***Gandhagam* A.F** | ***Padikaram* B.F** | ***Padikaram* A.F** | **Seena *rasa chendhuram*** | |
| **Appearance of the sample** | Yellow in colour | Yellow in colour | White in colour | White in colour | Black in colour | |
| **Solubility:**<br>**a.** A little of the sample is shaken well with distilled water.<br>**b.** A little of the sample is Shaken well with con. Hcl andn. H2SO4. | Sparingly soluble | Sparingly soluble | Soluble | soluble | Sparingly soluble | Presence of Silicate in *Gandhagam* and *Seenarasachendhuram*<br>Absence of silicate in *Padikaram* |
| **Action of Heat:**<br>A small amount of | No white | No white | No | No | No white | Absence of |

| | | | | | | |
|---|---|---|---|---|---|---|
| the sample is taken in a dry test tube and heated gently first and then strong. | fumes evolves | fumes evolves | white fumes evolves | white fumes evolves | fumes evolves | Carbonate |
| **Flame Test:**<br>A small amount of the sample is made into a paste with con.Hcl in a watch glass and introduced into non-luminous part of the Bunsen flame. | No appearance of bluish green flame | No appearance of bluish green flame | No appearance of bluishgreen flame | No appearance of bluishgreen flame | No appearance of bluishgreen flame | Absence of Copper |
| **Ash Test:**<br>A filter paper is soaked into a mixture of sample and cobalt nitrate solution and introduced into the Bunsen flame and ignited. | Appearance of yellow colour flame | Appearance of yellow colour flame | Appearance of yellow colour flame | Appearance of yellow colour flame | Appearance of yellow colour flame | Presence of Sodium |

## 4.3 QUANTITATIVE ANALYSIS

### 4.3.1 ICP-OES

### INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY

### INTRODUCTION

Inductively coupled plasma optical emission spectrometry (ICP-OES), is an analytical technique used for the detection of trace metals. It is a type of emission spectroscopy that uses the inductively coupled plasma to produce excited atoms and ions that emit electromagnetic radiation at wavelengths characteristic of a particular element. The intensity of this emission is indicative of the concentration of the element within the sample.

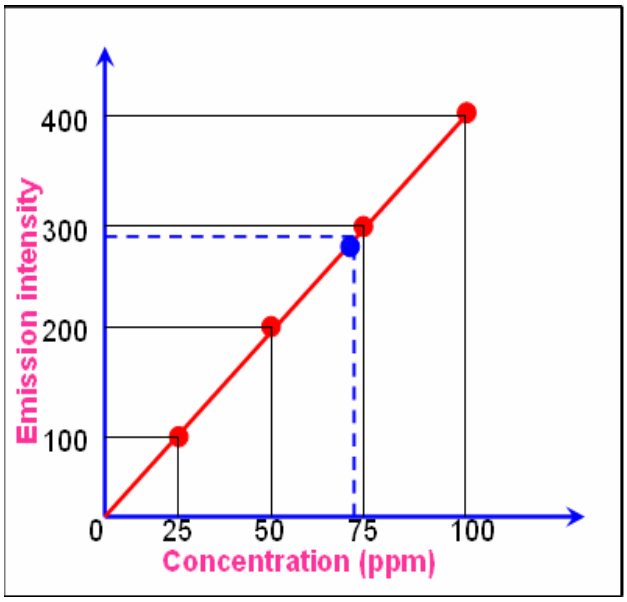
### PRINCIPLE

A Perkin-Elmer Optima ICP spectrometer is used for routine ICP-OES analysis. First, a high-energy radio frequency field is impinged upon a stream of argon gas. Then, a spark is used to ionize the argon gas, which forms sustained plasma due to inductive coupling with the high energy radio frequency field and the continuous supply of fresh argon to the plasma torch. This plasma has solutions passed into it in the form of a fine aerosol. The aerosol is dried, the dried particles broken apart, and the individual elements

are excited by interaction with the excited state argon in the plasma. As each atom returns to its ground state from the excited state, they emit light at wavelengths characteristic of the elements from which they originate. The emission intensity for each element is monitored for each standard solution and a calibration curve of emission intensity versus element concentration can be constructed.

**EXTRACTION OF INFORMATION**

Obtaining qualitative information, i.e., what elements are present in the sample, involves identifying the presence of emission at the wavelengths characteristic of the elements of interest. Obtaining quantitative information, i.e., how much of an element is in the sample, can be accomplished using plots of emission intensity versus concentration called calibration curves. Typical calibration graph is illustrated bellow.




Typical ICP Calibration curve

**Experimental Procedure: Done at SAIF, IIT Madras, Chennai-36**

Perkin Elmer Oplima 5300DV

40 M Hz RF generator;

Range: 165-782 nm;

Detection limit: Up to ppm level using SCD detector

**SAMPLE PREPARATION – Microwave Digestion**

- Weigh 0.25g of test sample and transfer into a liner provided with the instrument.
- Slowly add 9ml of Nitric acid or Sulphuric acid such that no piece of sample sticks on the slides.
- Mix thoroughly and allow reacting for few minutes.
- Place the liner in the vessel jacket.
- Close the screw cap hand-tight in clockwise direction.
- Seal the vessel and place in the rotor fixed in microwave.
- Set temperature to 180°C for 5 minutes; hold at 180°C for least 10 minutes.
- Allow the vessels to cool down to a vessel interior temperature below 60°C and to a vessel surface temperature (IR) below 50°C before removing the rotor.
- The digested sample was made up to 100ml with millipore water.
- If visible insoluble particles exist, solution could be filtered through whatmann filter paper.
- Transfer the digested solution into plastic containers and label them properly.

.

### 4.3.2 FOURIER TRANSFORM - INFRA RED SPECTROSCOPY

***PERKIN ELMER – SPECTRUM ONE***

**Introduction**

Vibrational spectroscopy is an extremely useful tool in the elucidations of molecular structure. The spectral bands can be assigned to different vibrational modes of the molecule. The various functional groups present in the molecule can be assigned by a comparison of the spectra with characteristic functional group frequencies. As the positions of the bands are directly related to the strength of the chemical bond, a large number of

investigations including intermolecular interactions, phase transitions and chemical kinetics can be carried out using this branch of spectroscopy.

In IR spectroscopy, the resonance absorption is made possible by the change in dipole moment accompanying the vibrational transition. The Infrared spectrum originates from the vibrational motion of the molecule. The vibrational frequencies are a kind of fingerprint of the compounds. This property is used for characterization of 60 organic, inorganic and biological compounds. The band intensities are proportional to the concentration of the compound and hence qualitative estimations are possible. The IR spectroscopy is carried out by using Fourier transform technique.

**Principle:**

Infra red spectroscopy involves study of the interaction of electromagnetic radiation with matter. Due to this interaction, electromagnetic radiation characteristic of the interacting system may be absorbed (or emitted). The experimental data consist of the nature (frequency of wave length) and the amount (intensity) of the characteristic radiation absorbed or emitted. These data are correlated with the molecular and electronic structure of the substance and with intra- and inter molecular interactions.

FT-IR spectroscopy is used primarily for qualitative and quantitative analysis of organic compounds, and also for determining the chemical structure of inorganic materials. The region between 500-4000 wave numbers is referred to as the finger print region. Absorption bands in this region are generally due to **intra molecular** phenomena and are highly specific for each material. The specificity if these bands allow computerized data searches to be performed against reference libraries to identify a material.

**Table of Characteristic IR Absorptions:**

| **Frequency, cm–1** | **Bond** | **Functional group** |
|---|---|---|
| 3640–3610 (s, sh) | O–H stretch, | free hydroxyl alcohols, phenols |
| 3500–3200 (s,b) | O–H stretch, H–bonded | alcohols, phenols |
| 3400–3250 (m) | N–H stretch | primary, secondary amines, amides |
| 3300–2500 (m) | O–H stretch | carboxylic acids |
| 3330–3270 (n, s) | –C(triple bond)C–H: C–H stretch | alkynes (terminal) |
| 3100–3000 (s) | C–H stretch | Aromatics |

| | | |
|---|---|---|
| 3100–3000 (m) | =C–H stretch | Alkenes |
| 3000–2850 (m) | C–H stretch | Alkanes |
| 2830–2695 (m) | H–C=O: C–H stretch | Aldehydes |
| 2260–2210 (v) | C(triple bond)N stretch | Nitriles |
| 2260–2100 (w) | –C(triple bond)C– stretch | Alkynes |
| 1760–1665 (s | C=O stretch | carbonyls (general) |
| 1760–1690 (s) | C=O stretch | carboxylic acids |
| 1750–1735 (s) | C=O stretch | esters, saturated aliphatic |
| 1740–1720 (s) | C=O stretch | aldehydes, saturated aliphatic |
| 1730–1715 (s) | C=O stretch | alpha,beta–unsaturated esters |
| 1715 (s) | C=O stretch | ketones, saturated aliphatic |
| 1710–1665 (s) | C=O stretch | alpha,beta–unsaturated aldehydes, ketones |
| 1680–1640 (m) | –C=C– stretch | Alkenes |
| 1650–1580 (m) | N–H bend | primary amines |
| 1600–1585 (m) | C–C stretch (in–ring) | Aromatics |
| 1550–1475 (s) | N–O asymmetric stretch | nitro compounds |
| 1500–1400 (m) | C–C stretch (in–ring) | Aromatics |
| 1470–1450 (m) | C–H bend | Alkanes |
| 1370–1350 (m) | C–H rock | Alkanes |
| 1360–1290 (m) | N–O symmetric stretch | nitro compounds |
| 1335–1250 (s) | C–N stretch | aromatic amines |
| 1320–1000 (s) | C–O stretch | alcohols, carboxylic acids, esters, ethers |
| 1300–1150 (m) | C–H wag (–CH2X) | alkyl halides |
| 1250–1020 (m) | C–N stretch | aliphatic amines |

| | | |
|---|---|---|
| 1000–650 (s) | =C–H bend | Alkenes |
| 950–910 (m) | O–H bend | carboxylic acids |
| 910–665 (s, b) | N–H wag | primary, secondary amines |
| 900–675 (s) | C–H "oop" | Aromatics |
| 850–550 (m) | C–Cl stretch | alkyl halides |
| 725–720 (m) | C–H rock | Alkanes |
| 700–610 (b, s) | –C(triple bond)C–H: C–H bend | Alkynes |

**Sampling techniques:**

There are a variety of techniques for sample preparation depending on the physical form of the sample to be analyzed.

Solid : KBr or Nujol mull method.

Liquid : CsI / TlBr Cells

Gas : Gas cells

**Experimental Procedure: Done at SAIF, IIT Madras, Chennai-36**

**KBr Method**

- The sample was grounded using- an agate mortar and pestle to give a very fine powder.
- The finely powder sample was mixed with about 100mg dried KBr salt.
- The mixture was then pressed under hydraulic press using a die to yield a transparent disc ( measure about 13mm diameter and 0.3mm in thickness), through which the beam of spectrometer passed.

### 4.3.3 X-Ray Fluorescence (XRF)

An X-ray fluorescence (XRF) spectrometer is an x-ray instrument used for routine, relatively non-destructive chemical analyses of rocks, minerals, sediments and fluids. It works on wavelength-dispersive spectroscopic principles that are similar to an electron microprobe (EPMA). However, an XRF cannot generally make analyses at the small spot sizes typical of EPMA work (2-5 microns), so it is typically used for bulk analyses of

larger fractions of geological materials. The relative ease and low cost of sample preparation, and the stability and ease of use of x-ray spectrometers make this one of the most widely used methods for analysis of major and trace elements in rocks, minerals, and sediment.

**Fundamental Principles of X-Ray Fluorescence (XRF)**

The XRF method depends on fundamental principles that are common to several other instrumental methods involving interactions between electron beams and x-rays with samples, including: X-ray spectroscopy (e.g., SEM - EDS), X-ray diffraction (XRD), and wavelength dispersive spectroscopy. Analysis of major and trace elements in geological materials by x-ray fluorescence is made possible by the behavior of atoms when they interact with radiation. When materials are excited with high-energy, short wavelength radiation (e.g., X-rays), they can become ionized. If the energy of the radiation is sufficient to dislodge a tightly-held inner electron, the atom becomes unstable and an outer electron replaces the missing inner electron. When this happens, energy is released due to the decreased binding energy of the inner electron orbital compared with an outer one. The emitted radiation is of lower energy than the primary incident X-rays and is termed fluorescent radiation. Because the energy of the emitted photon is characteristic of a transition between specific electron orbitals in a particular element, the resulting fluorescent X-rays can be used to detect the abundances of elements that are present in the sample.

**Procedure**

The analysis of major and trace elements in geological materials by XRF is made possible by the behavior of atoms when they interact with X-radiation. An XRF spectrometer works because if a sample is illuminated by an intense X-ray beam, known as the incident beam, some of the energy is scattered, but some is also absorbed within the sample in a manner that depends on its chemistry. The incident X-ray beam is typically produced from a Rh target, although W, Mo, Cr and others can also be used, depending on the application.

When this primary X-ray beam illuminates the sample, it is said to be excited. The excited sample in turn emits X-rays along a spectrum of wavelengths characteristic of the types of atoms present in the sample. How does this happen? The atoms in the sample absorb X-ray energy by ionizing, ejecting electrons from the lower (usually K and L) energy levels. The ejected electrons are replaced by electrons from an outer, higher energy orbital. When this happens, energy is released due to the decreased binding energy of the inner electron orbital compared with an outer one. This energy release is in the form of emission of characteristic X-rays indicating the type of atom present. If a sample has many elements present, as is typical for most minerals and rocks, the use of a Wavelength Dispersive Spectrometer much like that in an EPMA allows the separation of a complex emitted X-ray spectrum into characteristic wavelengths for each element present. Various types of detectors (gas flow proportional and scintillation) are used to measure the intensity of the emitted beam. The flow counter is commonly utilized for measuring long wavelength (>0.15 nm) X-rays that are typical of K spectra from elements lighter than Zn. The scintillation detector is commonly used to analyze shorter wavelengths in the X-ray spectrum (K spectra of element from Nb to I; L spectra of Th and U). X-rays of intermediate wavelength (K spectra produced from Zn to Zr and L spectra from Ba and the rare earth elements) are generally measured by using both detectors in tandem. The intensity of the energy measured by these detectors is proportional to the abundance of the element in the sample. The exact value of this proportionality for each element is derived by comparison to mineral or rock standards whose composition is known from prior analyses by other techniques.

X-Ray fluorescence is particularly well-suited for investigations that involve

- bulk chemical analyses of major elements (Si, Ti, Al, Fe, Mn, Mg, Ca, Na, K, P) in rock and sediment

- bulk chemical analyses of trace elements (in abundances >1 ppm; Ba, Ce, Co, Cr, Cu, Ga, La, Nb, Ni, Rb, Sc, Sr, Rh, U, V, Y, Zr, Zn) in rock and sediment - detection limits for trace elements are typically on the order of a few parts per million

X-ray fluorescence is limited to analysis of

- relatively large samples, typically > 1 gram
- materials that can be prepared in powder form and effectively homogenized
- materials for which compositionally similar, well-characterized standards are available
- materials containing high abundances of elements for which absorption and fluorescence effects are reasonably well understood

In most cases for rocks, ores, sediments and minerals, the sample is ground to a fine powder. At this point it may be analyzed directly, especially in the case of trace element analyses. However, the very wide range in abundances of different elements, especially iron, and the wide range of sizes of grains in a powdered sample, makes the proportionality comparison to the standards particularly troublesome. For this reason, it is common practice to mix the powdered sample with a chemical flux and use a furnace or gas burner to melt the powdered sample. Melting creates a homogenous glass that can be analyzed and the abundances of the (now somewhat diluted) elements calculated.

**Strengths**

X-Ray fluorescence is particularly well-suited for investigations that involve:

- bulk chemical analyses of major elements (Si, Ti, Al, Fe, Mn, Mg, Ca, Na, K, P) in rock and sediment
- bulk chemical analyses of trace elements (>1 ppm; Ba, Ce, Co, Cr, Cu, Ga, La, Nb, Ni, Rb, Sc, Sr, Rh, U, V, Y, Zr, Zn) in rock and sediment.

**Experimental Procedure Done at Sastra University, Tanjore.**

### 4.3.4 HR SEM-METHODOLOGY

**INTRODUCTION**

An SEM is essentially a high magnification microscope, which uses a focused scanned electron beam to produce images of the sample, both top-down and, with the necessary sample preparation, cross-sections. The primary electron beam interacts with the sample in a number of key ways:-

- Primary electrons generate low energy secondary electrons, which tend to emphasize the topographic nature of the specimen.
- Primary electrons can be backscattered which produces images with a high degree of atomic number (Z) contrast.
- Ionized atoms can relax by electron shell-to-shell transitions, which lead to either X-ray emission or Auger electron ejection. The X-rays emitted are characteristic of the elements in the top few μ m of the sample.

The SEM is carried out by using FEI-Quanta FEG 200-High Resolution Instrument.

**Resolution** **:** 1.2 nm gold particle separation on a carbon substrate

**Magnification** **:** From a min of 12 X to greater than 1, 00,000 X

**Application** **:** To evaluate grain size, particle size distributions, material homogeneity and inter metallic distributions.

**Experimental Procedure: Done at SAIF, IIT Madras, Chennai-36.**

**SAMPLE PREPARATION:**

Sample preparation can be minimal or elaborate for SEM analysis, depending on the nature of the samples and the data required. Minimal preparation includes acquisition of a sample that will fit into the SEM chamber and some accommodation to prevent charge build-up on electrically insulating samples. Most electrically insulating samples are coated with a thin layer of conducting material, commonly carbon, gold, or some other metal or alloy. The choice of material for conductive coatings depends on the data to be acquired:

carbon is most desirable if elemental analysis is a priority, while metal coatings are most effective for high resolution electron imaging applications. Alternatively, an electrically insulating sample can be examined without a conductive coating in an instrument capable of "low vacum" operation.

## 4.4 TOXICOLOGICAL EVALUATION OF SEENA RASA CHENDHURAM

### SCOPE OF WORK

Preclinical drug development is a stage that begins before clinical trials during which important safety and pharmacology data are collected. Regulatory toxicity studies are conducted in animals to identify possible hazards from which an assessment of risk to humans is made by extrapolation. The choice of animal species is based on the similarities of its metabolism to humans

The goals of the non clinical safety evaluation includes

- Categorization of toxic effects with respect to target organs, dose dependence, relationship to exposure and potential reversibility. This information is important for the estimation of an initial safe starting dose for the human trial.
- The identification of specific parameters for clinical monitoring for potential adverse effect.

# Systemic toxicity studies:

## Single dose study

Single dose studies (acute toxicity) in animals are essential for any pharmaceutical products intended for human use. The information obtained from these studies is useful in choosing doses for repeated dose studies, providing preliminary identification of target organs of toxicity and occasionally revealing delayed toxicity. Acute toxicity studies may also aid in the selection of starting doses for phase I human studies and provide information relevant to acute over dosing in humans.

### Repeated dose systemic toxicity studies

The primary goal of repeated dose toxicity studies is to characterize the toxicological profile of the cell compound following repeated administration. This includes identification of potential target organs of toxicity and exposure /response relationship and may include the potential reversibility of toxic effects. This information should be part of

the safety assessment to support the conduct of human clinical trials and the approval of marketing authorization

**WORK PLAN:**

The following studies were carried out on seena rasa chendhuram:

- Acute oral toxicity study (OECD guidelines – 423)
- 28 days Repeated oral toxicity study (OECD guidelines – 407)

The toxicity studies were evaluated after getting permission from the Institutional Animal Ethical Committee.

**IAEC PROTOCOL NO**: **1248/ac/09/CPCSEA/4-39/2O11.**

### 4.4.1 ACUTE TOXICITY STUDY OF SEENA RASA CHENDHURAM

**INTRODUCTION:**

1. The acute toxic class method is a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgement on the acute toxicity of the test substance. This procedure is reproducible, uses very few animals and is able to rank substances in a similar manner to the other acute toxicity testing methods. The acute toxic class method is based on biometric evaluations with fixed doses, adequately separated to enable a substance to be ranked for classification purposes and hazard assessment.

1. In principle, the method is not intended to allow the calculation of a precise LD50, but does allow for the determination of defined exposure ranges where lethality is expected since death of a proportion of the animals is still the major endpoint of this test. The method allows for the determination of an LD50 value only when at least two doses result in mortality higher than 0% and lower than 100%. The use of a selection of pre-defined doses, regardless of test substance, with classification explicitly tied to number of animals observed in different states improves the opportunity for laboratory to laboratory reporting consistency and repeatability.

## PRINCIPLE OF THE TEST

It is the principle of the test that, based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex (normally females). Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.;

− no further testing is needed,

− dosing of three additional animals, with the same dose

− dosing of three additional animals at the next higher or the next lower dose level

## METHODOLOGY

### 1. Selection of animal species

The preferred rodent species is the rat, although other rodent species may be used. Nulliparous and non-pregnant females with the strain of Wistar Albino rats were selected. Each animal, at the commencement of its dosing, was 6 weeks old and its weight lies in between 150-200g.

### 2. Housing and feeding conditions:

The temperature in the experimental animal room was 22ºC (±3ºC). Although the relative humidity was at least 30% and preferably not exceed 70% other than during room cleaning the aim was 50-60%. Lighting was artificial, the sequence being 12 hours light, 12 hours dark. For feeding, pellet diet with unlimited supply of RO water was used. Animals were group-caged by dose, but the number of animals per cage did not interfere with clear observations of each animal.

### Administration of doses:

*Seena rasa chendhuram* suspended in 10% Aqueous Tween 80 with vigorous mixing and was administered to the groups of Wistar Albino rats in a single oral dose with different dose levels by garage using a feeding needle. The control group received an equal volume of the vehicle. Animals were fasted prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. Three animals are used for each step. The dose level of 5, 50, 300 and 2000 mg/kg body weight was

administered stepwise. After the substance has been administered, food was withheld for a further 3-4 hours. The principle of laboratory animal care was followed. Observations were made and recorded systematically and continuously observed as per the guideline after substance administration. The visual observations included skin changes, mobility, aggressiveness, sensitivity to sound and pain and respiratory movements. They were deprived of food, but not water 12 h prior to the administration of the test suspension. Finally, the number of survivors was noted after 24 h and these animals were then maintained for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

**3. Test substance and Vehicle:**

The *Seena rasa chendhuram* is freely soluble in the suspension made with tween80 solution and water for dose accuracy and easy administration in animals.

**4. Justification for choice of vehicle:**

The vehicle selected as per the standard guideline which is pharmacologically inert and easy to employ for new drug development and evaluation technique.

**5. Test animals and Test conditions:**

Sexually mature female sex Wistar albino rats (150-200g) were obtained from Srinivasa animal laboratory, Bangalore. All the animals were kept at animal house in National Institute of Siddha, Chennai under standard environmental condition (22±3°C). The animals had free access to water and standard pellet diet (Sai meera foods, Bangalore). Rats were deprived of food but not water 12h prior to administration of the *Seena rasa chendhuram* .The principles of laboratory animal care were followed and the Department‘s ethical committee approved the use of the animals and the study design.

**Acute oral toxicity study (OECD guidelines – 423)**

| | | |
|---|---|---|
| Species and strain | : | Wistar Albino rat |
| Sex | : | Female |
| Age/Weight | : | 6 weeks/150-200 gm |
| Test guideline | : | OECD guidelines - 423 |
| Groups/treatment | : | Grouped by randomization |

Duration of exposure to the

*"Seena rasa chendhuram"* : Single dose

Study duration : 14 days

Number of animals : 3 females/group,

Route of administration : Oral

| **Groups** | **No of Rat** |
|---|---|
| Group I Vehicle control (Ghee) | 3 female |
| Group II Test drug – 5mg/kg b. wt | 3 female |
| Group III Test drug – 50 mg/kg b. wt | 3 female |
| Group IV Test drug – 300 mg/kg b. wt | 3 female |
| Group V Test drug – 2000 mg/kg b. wt | 3 female |

Acute oral toxicity of the formulations were evaluated in rats following OECD guideline - 423. Animals were divided into five groups, each group containing 3 females weighing 150 - 200g with age of 6 weeks. One group as control and the other four groups were treated with test drug at four different doses (5mg, 50mg, 300mg, 2000mg /kg.b.wt) by oral gavages.

**a. Behaviour:**

The animals were observed closely for behaviour in the first four hours which includes abnormal gait, aggressiveness, exophthalmos , ptosis , akinesia , catalepsy, convulsion, excitation, head twitches, lacrimation, loss of corneal reflex, loss of traction, piloerection reactivity of touch, salivation, scratching, sedation, stereotypes (chewing), stereotypes(head movements), stereotypes (sniffing), tremor and writhes, diarrhea, leathery, sleep and coma.

**b. Body weight :**

Body weights were recorded at day 1, 2, 7 and 14 of the study.

**c. Mortality :**

Animals were observed for mortality throughout the entire period.

**d. Gross necropsy:**

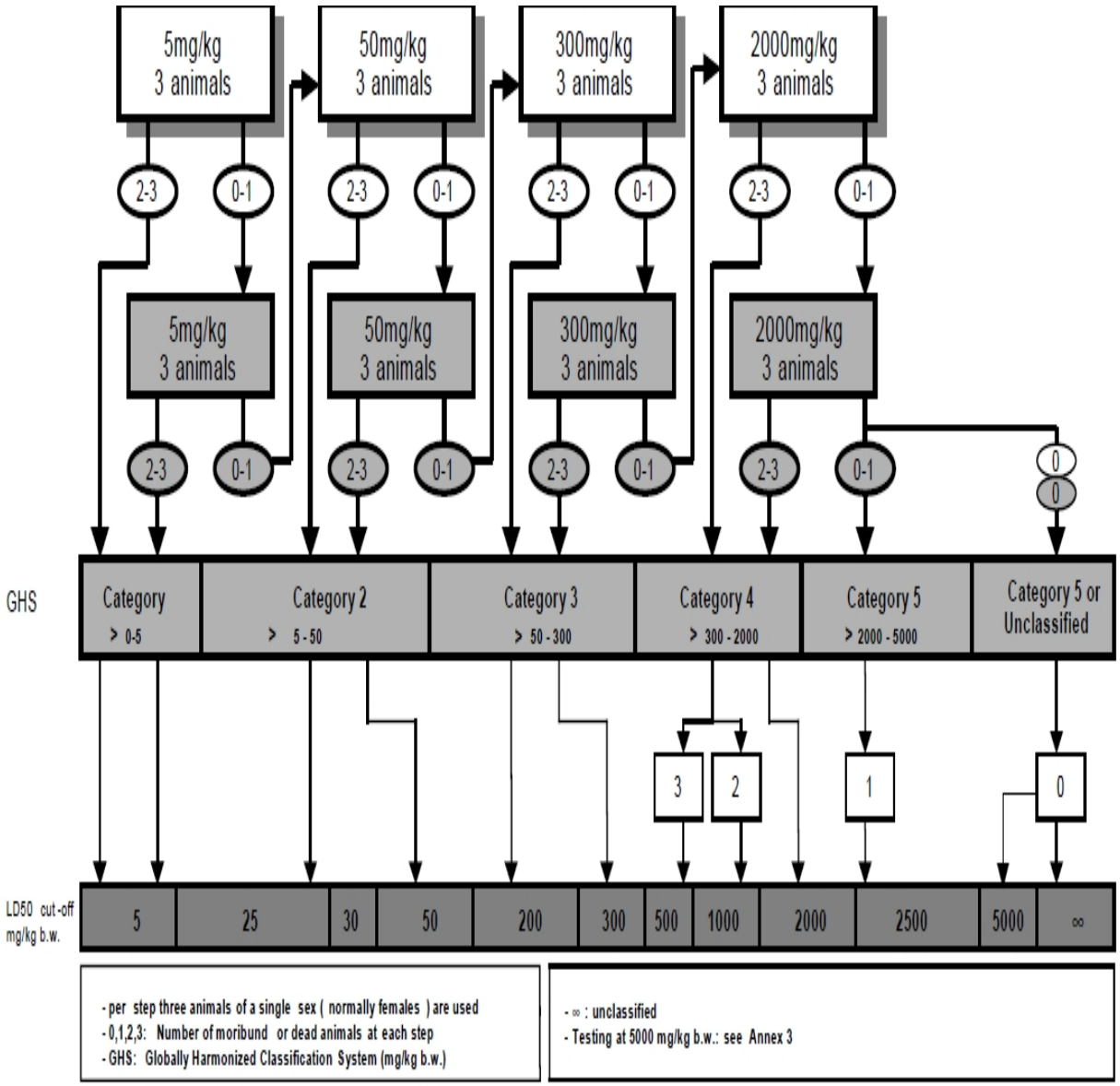
At the end of $14^{th}$ day animals were sacrificed for gross necropsy. It includes examination of the external surface of the body, all orifices, and organs like brain, lungs, heart, spleen, liver, stomach, kidneys, adrenals and sex organs of all animals.

**RESULTS**

Results are summarized as the number of animals displaying signs of toxicity, the number of animals found dead during the test or killed for humane reasons, time of death of individual animals, a description and the time course of toxic effects and reversibility, and necropsy findings.

**OECD-423**

**TEST PROCEDURE WITH A STARTING DOSE OF 5 MG/KG BODY WEIGHT**



### 4.4.2 28 DAYS REPEATED ORAL TOXICITY STUDY OF SEENA RASA CHENDHURAM

**OECD-407**

| | | |
|---|---|---|
| Species and strain | : | Wistar albino rats |
| Sex | : | Male and Female |
| Age/Weight | : | 6 weeks/150-200mg |
| Test guideline | : | OECD guidelines – 407 |
| Groups/treatment | : | Grouped by randomization |
| Duration | : | 28 days |
| Number of animals | : | 6/group (3/sex) |
| Route of administration | : | Oral |

| **Groups** | **No of Rats** |
|---|---|
| Group I Vehicle control (Ghee) | 6 (3male,3 female) |
| Group II test drug - low dose ( X – 9.36mg) | 6 (3male,3 female) |
| Group III test drug - Mid dose ( 5X – 46.8 mg) | 6 (3male,3 female) |
| Group IV test drug - High dose (10X – 93.6 mg) | 6 (3male,3 female) |

The study will be carried out as per OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents). The animals will be divided in four groups each group consist of 6 animals (3 males and 3 females). One group will serve as control and the other three groups for test drug at three different dose levels (low, mid and high) for 28 days.

**ANIMAL SOURCE:**

Test animals were obtained from The King Institute, Chennai and kept at animal house, National Institute of Siddha, Chennai. All the animals were kept under standard environmental condition (22 ± 3ºc) .The animals had free access to water and standard pellet diet (Sai Meera foods pvt. Ltd, Bangalore). The principles of laboratory animal care were followed.

**IDENTIFICATION OF ANIMAL:**

Animals were identified by cage number and individual marking on the fur of each animal with picric acid. Each animal was marked with picric acid. The females were nulliparous and non pregnant.

**HOUSING & ENVIRONMENT:**

The animals were allowed for an acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. The animals were housed in polypropylene cages provided with bedding of husk. Dark and light cycle each of 12 hours was maintained.

**ADMINISTRATION PERIOD:**

28 days

**DOSE SELECTION:**

Repeated oral toxicity study was carried out at different dose levels x (9.36mg), 5x (46.8mg), 10x (93.6mg).

**PREPARATION AND ADMINISTRATION OF DOSE**

*Seena rasa chendhuram* was suspended in 10% aqueous tween 80 solution. It was administered to groups I, II, IIIat dose levels of(x (9.36mg), 5x (46.8mg), 10x(93.6mg). The control animals were administered vehicle only. Administration was given orally by using an oral gavage once in daily for 28 days.

## OBSERVATIONS

### BODY WEIGHT:

During the study, Body weight of the animals was evaluated weekly once.

### FOOD AND WATER INTAKE:

Water and food consumption were calculated daily.

### MORTALITY:

Animals were observed for mortality daily.

### LABORATORY INVESTIGATIONS

**Collection of blood:**

Blood was collected in all overnight (12 hours) fasted rats through cardiac puncture method and it will be processed for below mentioned investigations.

**Laboratory test:**

Complete Haemogram

Renal function test

Liver function test

### NECROPSY

By the end of 28 days, the animals were sacrificed by excessive anesthesia. Animals were subjected to gross necropsy. Vital organs collected from the animals were subjected to histopathology.

## HISTOPATHOLOGY

Animals were subjected to histopathological investigations. Organs like heart, lungs, kidney, liver, spleen, stomach were collected from all animals and preserved in 10% buffered neutral formalin, sliced 5 or 6µm sections and it will be stained with hematoxylin and eosin, examined for histopathological changes.

## STATISTICAL ANALYSIS

Findings such as clinical sings of intoxication, body weight changes, food consumption, hematology, and biochemical parameters were subjected to one-way ANOVA followed by Dunnet "t" test using a computer software programme-INSTAT-V3 version.

# RESULTS

## PHYSICO-CHEMICAL PROPERTIES OF *SEENA RASA CHENDHURAM*

**Description : Black coloured fine powder**

**Nature : powdered material.**

**Table-1.**

**Colour characters of Seena Rasa Chenduram:**

| S No | Solvent used | Under ordinary light | Under ultra violet light |
|---|---|---|---|
| 1 | PM | Black | Black |

**PM-Powdered material**

**Table-2.**

**Physicochemical properties of Seena Rasa Chenduram:**

| S No. | Parameters | Values obtained (%w/w) | Heavy/ toxic metals | |
|---|---|---|---|---|
| 1 | Total ash value | 8.98 | Lead | BDL |
| 2 | Acid insoluble ash | 0.72 | Cadmium | BDL |
| 3 | Water soluble ash | 6.6 | Mercury | 3.241 mg/L |
| 4 | Moisture content | 10.24 | Arsenic | BDL |

**Table-3.**

**Colour, nature and percent yields of extracts of Seena Rasa Chenduram:**

| S.no. | Extract Solvents | Colour | Nature | % Yield(w/w) | SEM-Micro graph partical size range in Nm | pH |
|---|---|---|---|---|---|---|
| 1 | Water | Black | Solid | 49 | 25 – 75 nm | 8.3 – 8.5 |

**PHYSICO-CHEMICAL STANDARDISATION**

**Table- 4**

| S.No | TESTS | AS PER ANALYSIS |
|---|---|---|
| 1. | Description | Black coloured fine powder |
| 2. | Loss on Drying at 105°C | 7.83% |

## QUALITATIVE ANALYSIS

### TABLE -5

| S.No | PROCEDURES | GANDHAGAM | | PADIKARAM | | SEENA RASA CHENDHURAM |
|---|---|---|---|---|---|---|
| | | B.P | A.P | B.P | A.P | |
| 1. | Test for Ammonium | _ | _ | + | + | + |
| 2. | Test for Sodium | _ | _ | _ | _ | _ |
| 3. | Test for Magnesium | _ | _ | _ | _ | _ |
| 4. | Test for Aluminium | _ | _ | _ | _ | _ |
| 5. | Test for Potassium | _ | _ | _ | _ | _ |
| 6. | Test for Calcium | _ | _ | _ | _ | _ |
| 7. | Test for Ferrous iron | + | + | + | + | + |
| 9. | Test for Zinc | _ | _ | + | + | _ |
| 10. | Test for Arsenic | _ | _ | _ | _ | _ |
| 11. | Test for Mercury | _ | _ | _ | _ | _ |
| 12. | Test for Lead | _ | _ | + | _ | + |

| S.No | PROCEDURES | GANDHAGAM | | PADIKARAM | | SEENA RASA CHENDHURAM |
|---|---|---|---|---|---|---|
| | | B.P | A.P | B.P | A.P | |
| 13 | Test for Sulphate | – | – | – | – | + |
| 14 | Test for Chloride | – | + | – | + | – |
| 15 | Test for Phosphate | – | – | – | – | – |
| 16 | Test for carbonate | – | – | – | – | – |
| 17 | Test for Flouride & Oxalate | – | – | + | – | – |

\+ Present

\- Absent

## TEST FOR OTHER CONSTITUENTS

| S.No | PROCEDURES | GANDHAGAM | | PADIKARAM | | SEENA RASA CHENDHURAM |
|---|---|---|---|---|---|---|
| | | B.P | A.P | B.P | A.P | |
| 1. | Test for Starch | – | – | – | – | – |
| 2. | Test for Reducing sugar | – | – | – | – | – |
| 3. | Test for Alkaloids | + | + | + | + | + |
| 4. | Test for Amino acids | – | – | – | – | – |
| 5. | Test for Tannic acids | – | | – | – | – |
| 6. | Test for type of compounds | – | – | – | + | – |

\+ Present

\- Absent

## RESULTS OF QUALITATIVE ANALYSIS

### BIO CHEMICAL ANALYSIS

The qualitative analysis of given sample of unpurified Gandhagam shows the presence of

silicate

Iron

alkaloid

The qualitative analysis of given sample of purified Gandhagam shows the presence of

chloride

silicate

Iron

alkaloid

The qualitative analysis of given sample of unpurified Padikaram shows the presence of

Fluride and oxalate

Lead

Iron

Zinc

Alkaloid

The qualitative analysis of given sample of purified Padikaram show the presence of

Chloride

Iron

Zinc

Alkaloid

Ammonium

The qualitative analysis of given sample of Seena rasa chendhuram shows the presence of

Sulphate

Iron

Lead

Ammonium

Alkaloid

Silicate

## QUANTITATIVE ANALYSIS

**RESULTS OF ICP-OES**

**Sample description : SEENARASA CHENDHURAM**

**Equipment used : PERKIN ELMER OPTIMA 5300 DV**

**TABLE- 6**

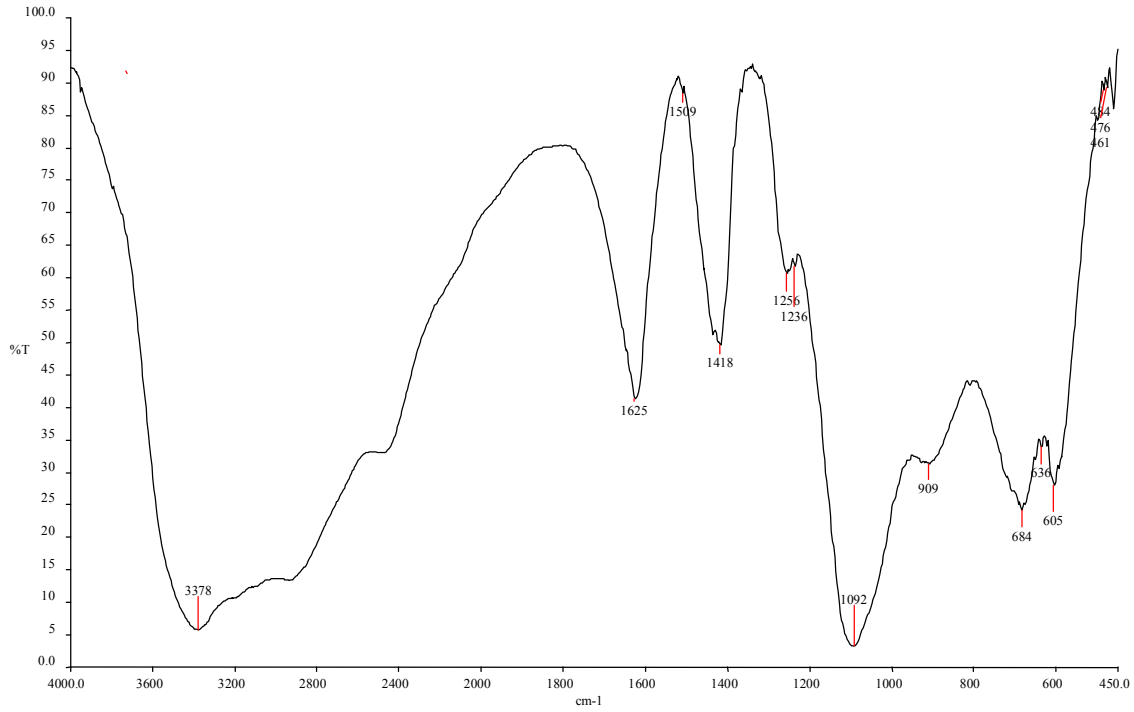
**INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY**

| S.NO | ANALYTE | WAVE LENGTH (nm) | OBSERVED RANGE IN PPM |
|---|---|---|---|
| | | | SEENA RASA CHENDHURAM |
| 1 | As | 193.696 | BDL |
| 2 | Ca | 317.933 | BDL |
| 3 | Cd | 226.502 | BDL |
| 4 | Hg | 253.652 | 3.241 |

| S.NO | ANALYTE | WAVE LENGTH (nm) | OBSERVED RANGE IN PPM |
|---|---|---|---|
| | | | SEENA RASA CHENDHURAM |
| | | | |
| 5 | P | 214.914 | 9.184 |
| 6 | Pb | 230.204 | BDL |

## FTIR ANALYSIS OF SEENARASA CHENDHURAM

**Fig.8**



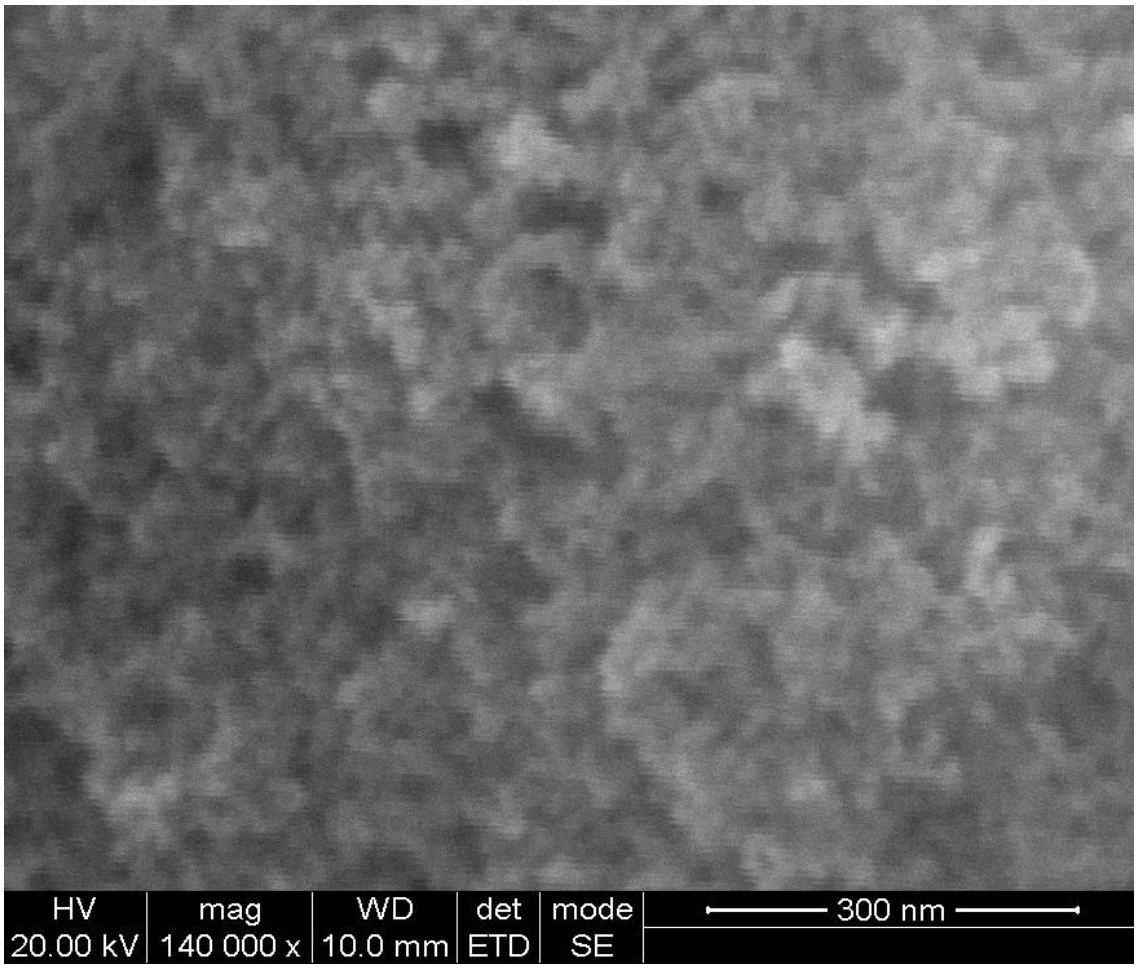
**FTIR ANALYSIS OF SEENA RASA CHENDHURAM**

**TABLE -7**

| Frequency, cm–1 | Bond | Functional group |
|---|---|---|
| 3378 | O–H stretch, H–bonded | alcohols, phenols |
| 1509 | N–O asymmetric stretch | nitro compounds |
| 1625 | N–H bend | primary amines |
| 1418 | C–C stretch (in–ring) | Aromatics |
| 1256 | C–O stretch | alcohols, carboxylic acids, esters, ethers |
| 1236 | C–N stretch | aliphatic amines |
| 1092 | C–O stretch | alcohols, carboxylic acids, esters, ethers |
| 909 | N–H wag | primary, secondary amines |
| 684 | C–Br stretch | alkyl halides |
| 636 | –C(triple bond)C–H: C–H bend | Alkynes |
| 605 | C–Br stretch | alkyl halides |

**HR SEM Analysis - Determination of particle size of Seena rasa chendhuram**

**Fig.9**




| S.no. | Extract Solvents | Colour | Nature | % Yield(w/w) | SEM-Micro graph partical size range in nm | pH |
|---|---|---|---|---|---|---|
| 1 | Water | Black | **Solid** | 49 | 25 – 75 nm | 8.3 – 8.5 |

# XRF RESULT OF SEENARASA CHENDHURAM

## Table - 8

| Formula | Z | Concentration | Status | Line 1 | Calc. concentration | Stat. error | LLD | Analyzed layer |
|---|---|---|---|---|---|---|---|---|
| O | 8 | 48.97 %XRF 0 | O KA1-HR | 1.512 | 12.4 % | 0.867 % | | 0.89 um |
| S | 16 | 31.37 % | XRF 0 | S KA1-HR-Tr | 31.37 | 0.144 % | 155.1 PPM | 10.0 um |
| Hg | 80 | 17.13 % | XRF 0 | Hg LA1-HR-Tr | 17.13 | 0.132 % | 348.9 PPM | 134 um |
| Al | 13 | 1.90 % | XRF 0 | Al KA1-HR-Tr | 1.90 | 0.940 % | 101.3 PPM | 3.7 um |
| Fe | 26 | 0.21 % | XRF 0 | Fe KA1-HR-Tr | 0.213 | 1.34 % | 45.2 PPM | 41 um |
| Si | 14 | 0.16 % | XRF 0 | Si KA1-HR-Tr | 0.161 | 3.40 % | 120.5 PPM | 5.2 um |
| Cl | 17 | 0.06 % | XRF 0 | Cl KA1-HR-Tr | 0.060 | 7.77 % | 142.3 PPM | 4.7 um |
| K | 19 | 0.05 % | XRF 0 | K KA1-HR-Tr | 0.046 | 7.07 % | 78.8 PPM | 7.4 um |
| Tl | 81 | 0.04 % | XRF 0 | Tl LA1-HR-Tr | 0.042 | 14.4 % | 103.3 PPM | 145 um |
| Mn | 25 | 0.04 % | XRF 0 | Mn KA1-HR-Tr | 0.0381 | 4.70 % | 53.6 PPM | 33 um |
| Ca | 20 | 0.03 % | XRF 0 | Ca KA1-HR-Tr | 0.034 | 7.90 % | 72.7 PPM | 9.6 um |
| As | 33 | 0.03 % | XRF 0 | As KA1-HR-Tr | 0.026 | 7.86 % | 30.4 PPM | 157 um |
| Ge | 32 | 99 PPM | XRF 0 | Ge KA1-HR-Tr | 0.00986 | 0.926 % | 266.5 PPM | 131 um |
| Cu | 29 | 60 PPM | XRF 0 | Cu KA1-HR-Tr | 0.006 | 12.3 % | 30.5 PPM | 75 umni9s 4 ele |

# Table - 9

| Formula | Z | Concentration | Status | Line 1 | Calc. concentration | Stat. error | LLD | Analyzed layer |
|---|---|---|---|---|---|---|---|---|
| SO3 | 16 | 78.32 % | XRF 1 | S KA1-HR-Tr | 78.32 | 0.144 % | 387.2 PPM | 10.0 um |
| Hg | 80 | 17.13 % | XRF 1 | Hg LA1-HR-Tr | 17.13 | 0.132 % | 348.9 PPM | 134 um |
| Al2O3 | 13 | 3.59 % | XRF 1 | Al KA1-HR-Tr | 3.59 | 0.940 % | 191.3 PPM | 3.7 um |
| SiO2 | 14 | 0.34 % | XRF 1 | Si KA1-HR-Tr | 0.344 | 3.40 % | 257.8 PPM | 5.2 um |
| Fe2O3 | 26 | 0.31 % | XRF 1 | Fe KA1-HR-Tr | 0.305 | 1.34 % | 64.6 PPM | 41 um |
| Cl | 17 | 0.06 % | XRF 1 | Cl KA1-HR-Tr | 0.060 | 7.77 % | 142.3 PPM | 4.7 um |
| K2O | 19 | 0.06 % | XRF 1 | K KA1-HR-Tr | 0.055 | 7.07 % | 95.0 PPM | 7.4 um |
| MnO | 25 | 0.05 % | XRF 1 | Mn KA1-HR-Tr | 0.0492 | 4.70 % | 69.2 PPM | 33 um |
| CaO | 20 | 0.05 % | XRF 1 | Ca KA1-HR-Tr | 0.048 | 7.90 % | 101.8 PPM | 9.6 um |
| Tl | 81 | 0.04 % | XRF 1 | Tl LA1-HR-Tr | 0.042 | 14.4 % | 103.3 PPM | 145 um |
| As2O3 | 33 | 0.03 % | XRF 1 | As KA1-HR-Tr | 0.034 | 7.86 % | 40.1 PPM | 157 um |
| GeO2 | 32 | 0.01 % | XRF 1 | Ge KA1-HR-Tr | 0.01421 | 0.926 % | 383.9 PPM | 131 um |
| CuO | 29 | 75 PPM | XRF 1 | Cu KA1-HR-Tr | 0.007 | 12.3 % | 38.2 PPM | 75 um |

# RESULTS OF TOXICITY STUDY

## RESULTS OF ACUTE ORAL TOXICITY STUDY

All the datas were summarized in the form of table (10) Showing the animals behavioural signs in control and test groups.

**At the dose level of 5 and 50 mg/kg body wt there were no abnormal signs were detected. No abnormality in necropsy findings.**

**At the dose level of 300mg/kg body wt animals show the following toxic signs on the 2nd after drug administration.**

EYES : Protruded

HINDLIMB : Extended

GRIP : Power decreased

FOOD INTAKE : Normal

WATER INTAKE: Normal

On 7th day nasal discharge was seen. Eyes were normal.

On 12th day animals show normal behavioural signs. And toxic signs were reversible.

On 14th day no abnormality in necropsy findings.

**At the dose level of 2000mg/kg body wt animals show the following toxic signs:**

EYES : Protruded

HINDLIMB : Extended

GRIP : Power decreased

FOOD INTAKE : Normal

WATER INTAKE: Normal

BODY WEIGHT: Normal

On examination generalized bulging seen.

Nasal bleeding seen.

Muscular Inco-ordination seen

Muscular spasm seen.

**Two animals found dead** on the day of administration.

**Necropsy findings of 2000mg/kg body weight dead animals:**

Lung congestion seen

Ulceration in stomach seen.

Remaining one animal in 2000mg group shows no abnormal findings in Necropsy.

This concludes that **LD50 cut-off** of Test drug is **1000mg/kg body weight** and it comes under **CAT - 4 (GHC)** as per the oecd 423 classification.

**RESULTS OF 28 DAYS REPEATED ORAL TOXICITY STUDY**

**Clinical signs:**

No abnormal behavioral signs were observed during the study period.

**Mortality:**

The test drug Seena rasa chendhuram did not cause any mortality in X(9.36), 5X(46.8) and 10X(93.6) dose levels and were considered as safe dose levels.

**Body Weight:**

Both control and test dose groups exhibited body weight gain throughout the administration period of 14 days Table (11).

**Food consumption:**

No difference in food intake of control and test group animals observed during the period of study. Table (12).

**Water Intake:**

No difference in water intake of control and test group animals observed during the period of study. Table (13)

**Hematological investigation:**

The results of hematological investigation conducted at the end of the study, test groups revealed no significant changes in values of different parameters, when compared with control group. The Hb count was slightly elevated in test groups, but statistically not significant when compared with control group. Table (14).

**Biochemical investigations:**

Biochemical investigations were conducted at the end of the study and the results were recorded. In test groups there was no significant elevation in the levels of biochemical parameters, when compared with the control group. And the values obtained were within normal biological limits. Table (15, 16, 17).

**Histopathology**

Gross pathological examination of animals doesn't reveal any abnormalities in control groups. The histopathological study of the organs such as heart, lungs, Spleen, Stomach and kidney were normal in control, X, and 5X and 10 X groups. Liver shows Lymphocyte infiltration in 10X groups.

## Histopathology report of *Seena rasa chendhuram*

**KIDNEY**

**Control:**

Specimen shows renal parenchyma with normal appearing cortex and medulla.

**Low dose:**

Section shows patent glomeruli .

Tubules on interstitium unremarkable.

**Mid dose:**

Section shows patent glomeruli

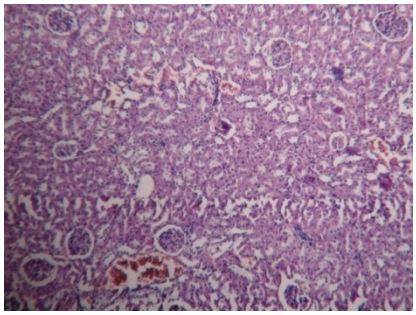
Tubules on interstitium unremarkable

**High dose:**

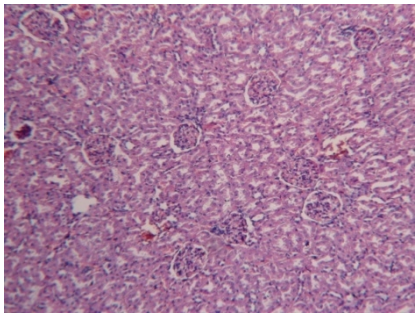
Section shows patent glomeruli .

Tubules on interstitium unremarkable.

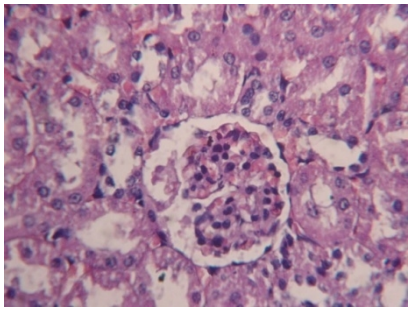
**KIDNEY**



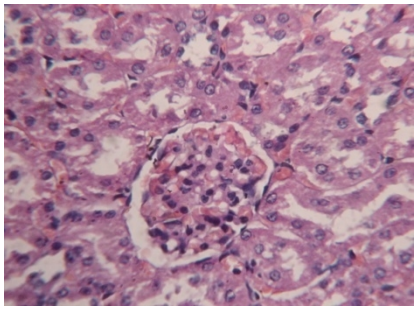
CONTROL



LOW DOSE



MID DOSE



HIGH DOSE

**LIVER**

**Control:**

Section from the liver shows normal appearing central veins with radiating hepatocytes, sinusoids, kuffer cells and portal triad.

**Low dose:**

Shows central veins surrounded by radiating cords of hepatocytes.

Sinusoids contain kupfer cells seen in between the rows of hepatocytes.

Portal triads appear normal.

**Mid dose:**

Shows central veins surrounded by radiating cords of hepatocytes

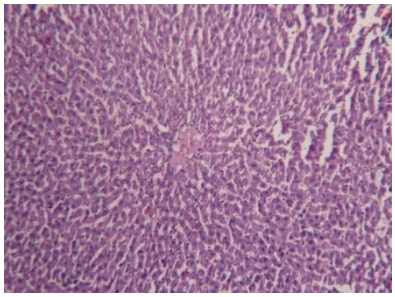
Portal triads appear normal.

**High dose:**

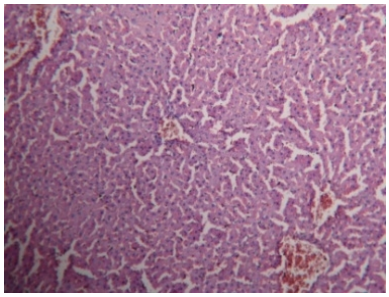
Shows central veins surrounded by radiating cords of hepatocytes

Portal tract shows lymphocyte infilteration

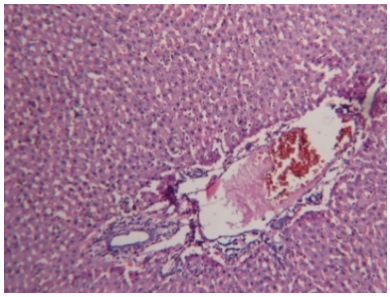
**LIVER**



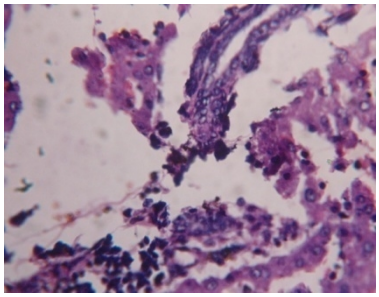
CONTROL



LOW DOSE



MID DOSE



HIGH DOSE(Portal triad with lymphoid infilterate)

SPLEEN

**Control:**

Shows white pulp germinal centers and central arterioles surrounded by congested red pulp.

**Low dose:**

Shows white pulp germinal centers and central arterioles surrounded by congested red pulp.

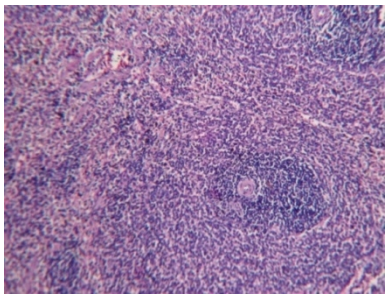
**Mid dose:**

Shows white pulp germinal centers and central arterioles surrounded by congested red pulp.

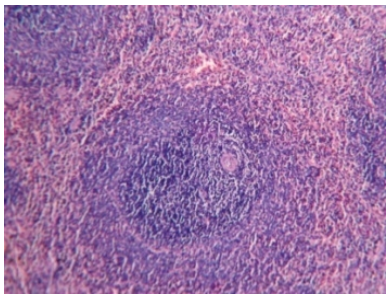
**High dose:**

Shows white pulp germinal centers and central arterioles surrounded by congested red pulp.

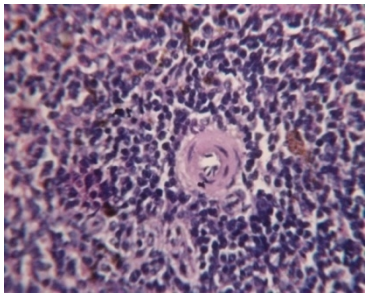
SPLEEN



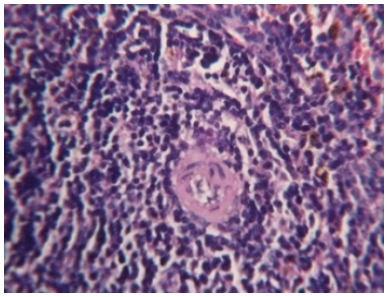
CONTROL



LOW DOSE



MID DOSE



HIGH DOSE

**HEART**

**Control:**

Sections show normal myocardial fibers and blood vessels.

**Low dose:**

Section shows normal appearing cardiac myocytes

**Mid dose:**

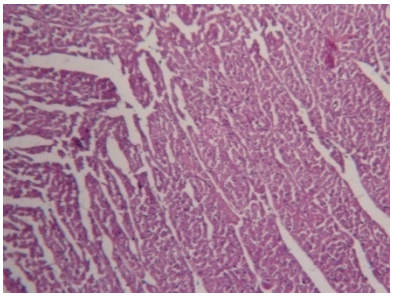
Section shows normal appearing cardiac myocytes.

Coronary artery is patent.

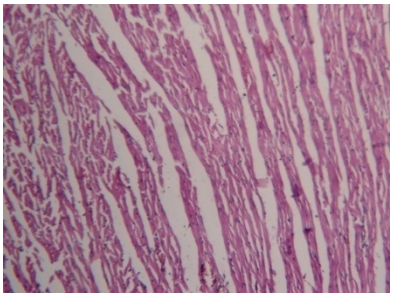
**High dose**:

Section shows normal appearing cardiac myocytes.

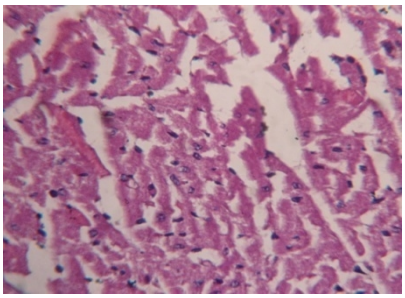
HEART



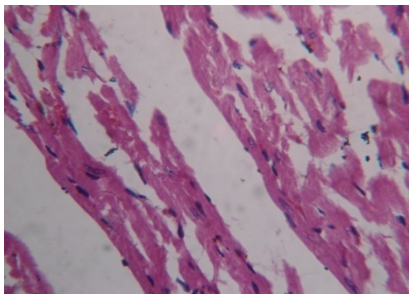
CONTROL



LOW DOSE



MID DOSE



HIGH DOSE

**LUNGS**

**Control:**

Section from the lung shows normal appearing bronchioles, alveoli, interstitium and blood vessels

**Low dose**:

Shows bronchioles and alveoli with focal lymphoid aggregates.

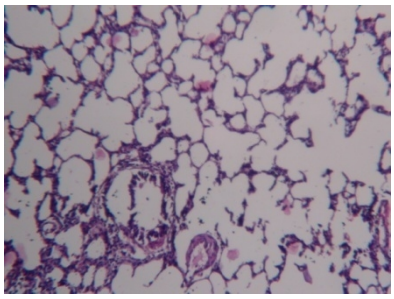
**Mid dose:**

Shows bronchioles and alveoli with focal lymphoid aggregates.

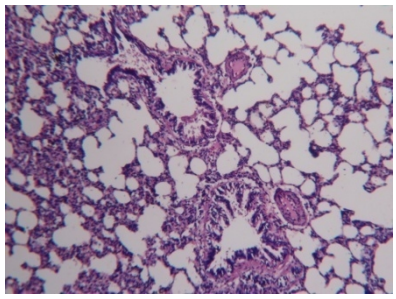
**High dose**:

Shows bronchioles and alveoli with focal lymphoid aggregates.

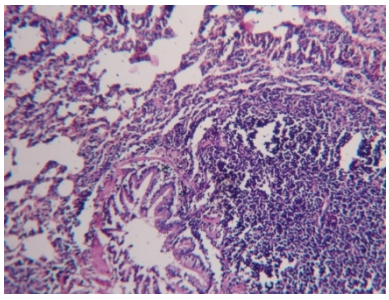
LUNGS



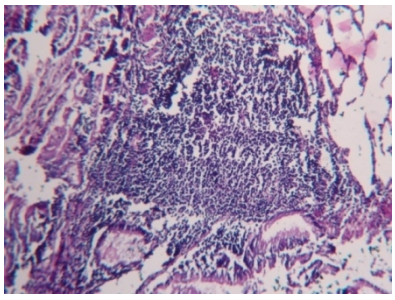
CONTROL



LOW DOSE



MID DOSE



HIGH DOSE

**STOMACH**

**Control:**

Shows gastric mucosa with mucosal glands lined by tall columnar cells

**Low dose:**

Shows gastric mucosa with mucosal glands lined by tall columnar cells

**Mid dose:**

Shows gastric mucosa with mucosal glands lined by tall columnar cells

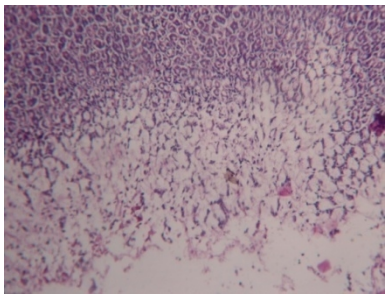
Muscularis mucosa and muscularis propriae appears normal

**High dose:**

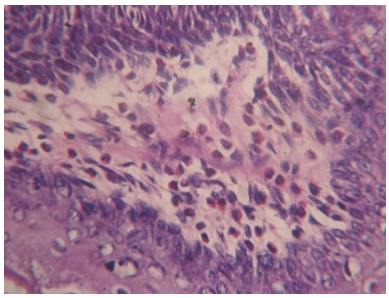
Shows gastric mucosa with mucosal glands lined by tall columnar cells.

Muscularis mucosa and muscularis propriae appears normal.

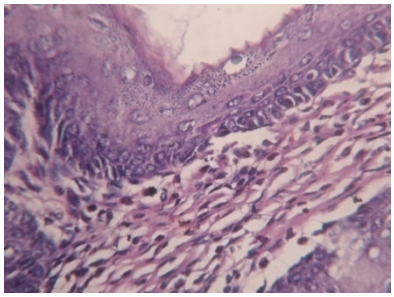
STOMACH



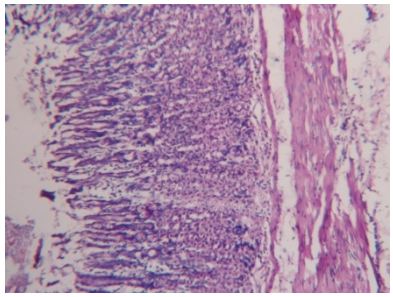
CONTROL



LOW DOSE



MID DOSE



HIGH DOSE

## TABLES AND CHARTS

## Acute oral toxicity:

**Table- 10: Dose finding experiment and its behavioral Signs of Toxicity**

| No | Dose mg/kg | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | 5 | + | - | - | + | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 50 | + | - | - | - | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 300 | + | - | - | + | + | + | - | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 2000 | + | - | - | + | + | + | - | + | - | - | - | - | - | - | - | - | + | - | - | - |

1.Alertness 2. Aggressiveness 3. Pile erection 4. Grooming 5. Gripping 6. Touch Response 7. Decreased Motor Activity 8. Tremors 9. Convulsions 10. Muscle Spasm 11. Catatonia 12. Muscle relaxant 13. Hypnosis 14. Analgesia 15.Lacrimation 16. Exophthalmos 17. Diarrhoea 18. Writhing 19. Respiration 20. Mortality

## 28 Days Repeated oral toxic study of *seena rasa chendhuram*

### BODY WEIGHT OF WISTAR ALBINO RATS GROUP EXPOSED TO

## *Seena rasa chendhuram*

**TABLE- 11**

| DOSE | BODY WEIGHT (gms/rat) | | | | |
|---|---|---|---|---|---|
| | **DAY 1** | **DAY 7** | **DAY 14** | **DAY 21** | **DAY 28** |
| **CONTROL** | 159±5.51 | 162.67±2.42 | 165.33±2.42 | 166.17±1.94 | 167.83±2.32 |
| **X** | 162.83±6.77 | 165±6.54 | 166.33±6.56 | 168±6.36 | 171.67±5.79 |
| **5X** | 171.67±6.62 | 173.83±3.67 | 175.50±6.06 | 176.67±5.92 | 178.17 ±5.91 |
| **10X** | 172.50±1.87 | 173.83±1.60 | 175.17±1.17 | 176.50±1.05 | 177.50±1.05 |
| **P VALUE** | >0.05 N.S | >0.05 N.S | >0.05 N.S | >0.05 N.S | >0.05 N.S |

Values are mean of 6 animals ± S.D, N.S-Non significance

**FOOD INTAKE OF WISTER ALBINO RATS GROUP EXPOSED TO**

## *seena rasa chendhuram*

**TABLE- 12**

| DOSE | FOOD INTAKE (gms/rat) | | | |
|---|---|---|---|---|
| | **DAY 1** | **DAY 7** | **DAY 14** | **DAY 28** |
| **CONTROL** | 31.16±3.31 | 38.83±5.91 | 40.16±4.07 | 41.47±5.42 |
| **X** | 32.74±3.27 | 34.31±4.17 | 38.25±3.68 | 39.76±3.41 |
| **5X** | 40.16±4.08 | 39.16±2.73 | 41.15±5.10 | 41.40±5.31 |
| **10X** | 35.25±3.5 | 35±2.60 | 40.16±3.54 | 40.51±4.73 |
| **P VALUE** | >0.05 N.S | >0.05 N.S | >0.05 N.S | >0.05 N.S |

Values are mean of 6 animals ± S.D, N.S-Non significance

**WATER INTAKE OF WISTER ALBINO RATS GROUP EXPOSED *seena rasa chendhuram***

**TABLE- 13**

| DOSE | WATER INTAKE (ml/rat) | | | |
|---|---|---|---|---|
| | **DAY 1** | **DAY 7** | **DAY 14** | **DAY 28** |
| **CONTROL** | 48.41±6.52 | 50.32±5.21 | 48±4.11 | 49±5.18 |
| **X** | 50.66±3.55 | 47.5±2.73 | 41.66±2.58 | 40.12±2.36 |
| **5X** | 48.33±6.05 | 49.33±3.67 | 43±3.48 | 40±3.21 |
| **10X** | 49.16±4.91 | 58.33±5.16 | 46.83±5.11 | 43.35±5.14 |
| **P VALUE** | >0.05 N.S | >0.05 N.S | >0.05 N.S | >0.05 N.S |

Values are mean of 6 animals ± S.D, N.S-Non significance

**EFFECT OF *seena rasa chendhuram* ON HEMATOLOGICAL PARAMETERS**

**TABLE- 14**

| INVESTIGATION | CONTROL | GROUP 1 | GROUP 2 | GROUP 3 | P VALUE |
|---|---|---|---|---|---|
| **Hb%** | 12.23 ±0.10 | 13.25±0.24 | 12.25±0.14 | 12.53±0.18 | >0.05 N.S |
| **Tc cells/ $mm^3$** | 9050±104.88 | 8966.67±121.11 | 8850±187.08 | 8216.67±147.20 | >0.05 N.S |
| **Polymorphs%** | 36.5±26.2 | 58±2.5 | 46.8±17.5 | 38.6±20.5 | >0.05 N.S |
| **Lymphocytes%** | 58±19.8 | 41.5±2 | 52.5±16.9 | 59.3±9.03 | >0.05 N.S |
| **Esinophils%** | 1.67±1.03 | 2.33±1.63 | 2.17±1.47 | 1.50±0.55 | >0.05 N.S |
| **Platelet** | 4.45±.75 | 3.26±0.39 | 3.23±0.22 | 3.26±0.23 | >0.05 N.S |
| **Monocyte** | 0.50±.55 | 0.33±.52 | 0.50±0.55 | 0.50±0.55 | >0.05 N.S |
| **TRBC cells/ $mm^3$** | 3.8±.3 | 6±1.6 | 5.8±1 | 6.5±2 | >0.05 N.S |
| **PCV** | 48.20 ± 1.88 | 43.89 ± 2.10 | 45.38 ± 1.66 | 44.10 ± 2.14 | >0.05 N.S |
| **MCH pg** | 18.18 ± 0.25 | 18.25 ± 1.45 | 18.39 ± 0.47 | 18.40 ± 0.10 | >0.05 N.S |
| **MCHC g/dl** | 30.56 ± 0.88 | 31.10 ± 0.41 | 30.44 ± 1.22 | 30.05 ± 0.44 | >0.05 N.S |
| **MCV (gl)** | 58.78 ± 4.36 | 55.38 ± 3.68 | 54.68 ± 2.11* | 55.36 ± 4.60* | >0.05 N.S |

Values are mean of 6 animals ± S.D, N.S-Non significance (*P<O.O5)

## BIO CHEMICAL PARAMETERS

## EFFECT OF *seena rasa chendhuram* ON BLOOD SUGAR AND LIPID PROFILE

**TABLE- 15**

| INVESTIGATION mg/dl | CONTROL | GROUP 1 | GROUP 2 | GROUP 3 | P VALUE |
|---|---|---|---|---|---|
| **B.Glucose** | 98.3±38.3 | 74.8±4.7 | 78.3±8.3 | 81.2±9.3 | >0.05 N.S |
| **T.Cholestrol** | 65.5±3.5 | 66.33±3.4 | 70.3±4.0 | 76±5.2 | >0.05 N.S |
| **T GL** | 135±13.1 | 103.3±14.1 | 111.4±20.5 | 121±5.2 | >0.05 N.S |
| **HDL** | 20±3.9 | 22.12±2.1 | 23.1±3.16 | 25.8±3.8 | >0.05 N.S |
| **LDL** | 17.9±2.0 | 22.43±4.1 | 24.4±4.2 | 25.3±3.3 | >0.05 N.S |
| **VLDL** | 25±2.2 | 21.7±3.04 | 22.1±4.2 | 23.8±1.7 | >0.05 N.S |

Values are mean of 6 animals ± S.D, N.S-Non significance

**EFFECT OF *seena rasa chendhuram* ON RENAL PARAMETERS**

**TABLE- 16**

| INVESTIGATION | CONTROL | GROUP 1 | GROUP 2 | GROUP 3 | P VALUE |
|---|---|---|---|---|---|
| **Urea mg/dl** | 27.7±14.8 | 33.7±4.0 | 23±7.2 | 25.8±2.4 | >0.05 N.S |
| **Creatinine mg/dl** | 0.64±.10 | 0.74±0.07 | 0.60±0.07 | 0.68±0.08 | >0.05 N.S |
| **Uric acid mg/dl** | 2.40±.08 | 2.26±.33 | 2.28±0.61 | 2.2±.49 | >0.05 N.S |
| **Calcium m.Eq/l** | 8.4±0.7 | 8.6±0.62 | 8.3±1.06 | 9.2±0.43 | >0.05 N.S |
| **Phosphorus m.Eq/l** | 2.78±.06 | 3.0±.58 | 2.6±0.89 | 3.4±0.50 | >0.05 N.S |

Values are mean of 6 animals ± S.D, N.S-Non significance

## EFFECT OF *seena rasa chendhuram* ON HEPATIC PARAMETERS

**TABLE- 17**

| INVESTIGATION | CONTROL | GROUP 1 | GROUP 2 | GROUP 3 | P VALUE |
|---|---|---|---|---|---|
| **T.Bilirubin mg/dl** | 0.7±.09 | 0.7±0.12 | 0.71±0.15 | 0.60±0.08 | >0.05 N.S |
| **Dir. Bilirubin mg/dl** | 0.2±0.07 | 0.3±0.05 | 0.34±0.08 | 0.24±0.04 | >0.05 N.S |
| **Ind. Bilirubin mg/dl** | 0.4±0.06 | 0.35±0.11 | 0.35±0.10 | 0.34±0.07 | >0.05 N.S |
| **SGOT U/L** | 53±6.9 | 71.7±23.46 | 50.1±5.0 | 62.1±18.80 | >0.05 N.S |
| **SGPT U/L** | 60±8.2 | 80.5±32.6 | 65.3±11.5 | 73.8±19.3 | >0.05 N.S |
| **Alk.ph U/L** | 140±7.6 | 141.6±15.0 | 147.3±18.1 | 143.3±8.03 | >0.05 N.S |
| **Total protein g/dl** | 6.5±0.39 | 7.09±0.36 | 6.75±0.61 | 6.66±0.29 | >0.05 N.S |
| **Albumin g/dl** | 3.03±0.33 | 3.38±0.23 | 3.4±0.33 | 3.48±0.31 | >0.05 N.S |
| **Globulin g/dl** | 3.46±0.07 | 3.54±0.43 | 3.38±0.39 | 3.0±0.22 | >0.05 N.S |

Values are mean of 6 animals ± S.D, N.S-Non significance

**CHART 1**

**THE MEAN WEIGHT OF CONTROL AND TREATED GROUPS**

**(REPEATED TOXIC STUDY)**

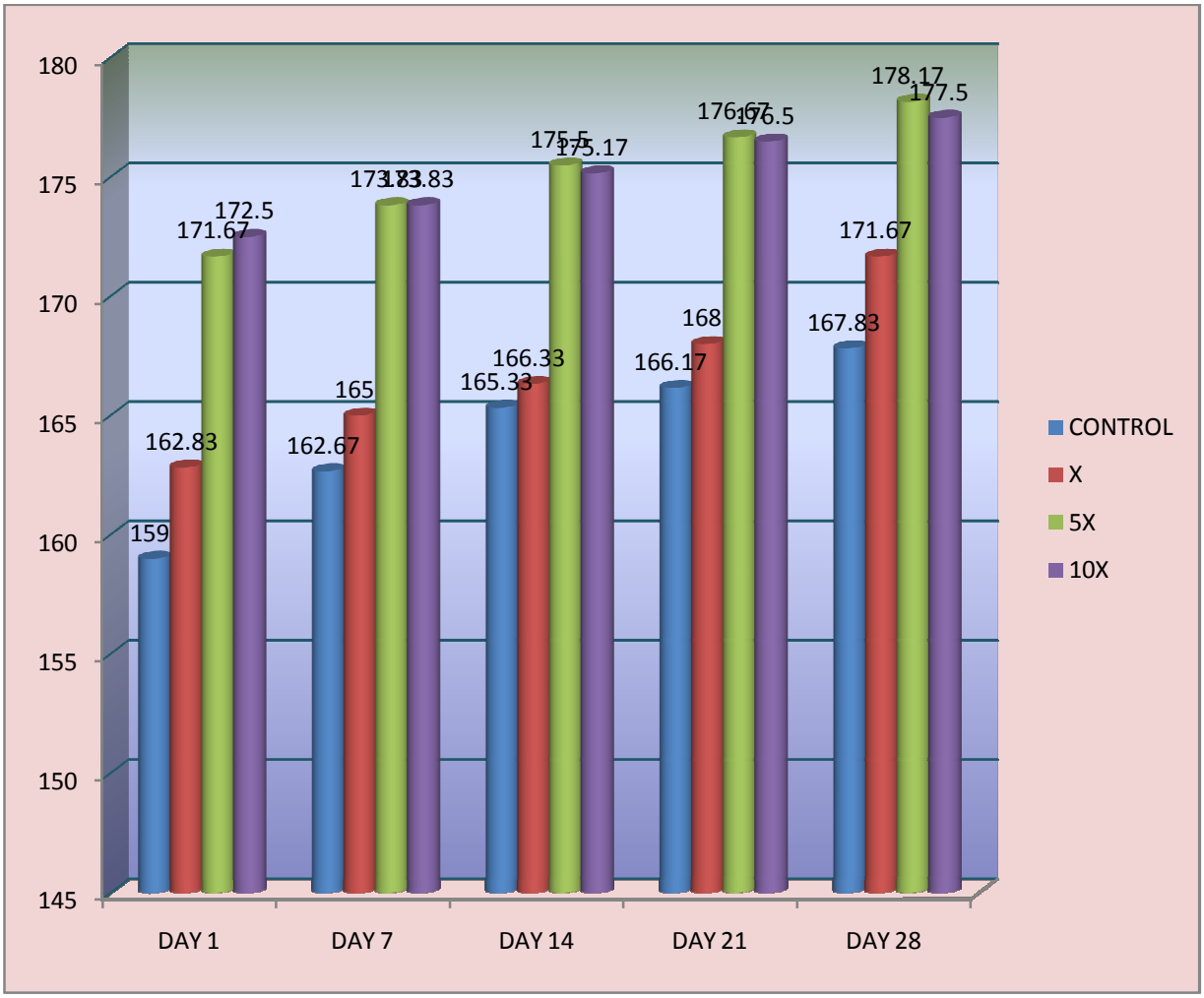


**CHART 2**

**THE AVERAGE INTAKE OF FOOD BY CONTROL**

**AND TREATED GROUPS**

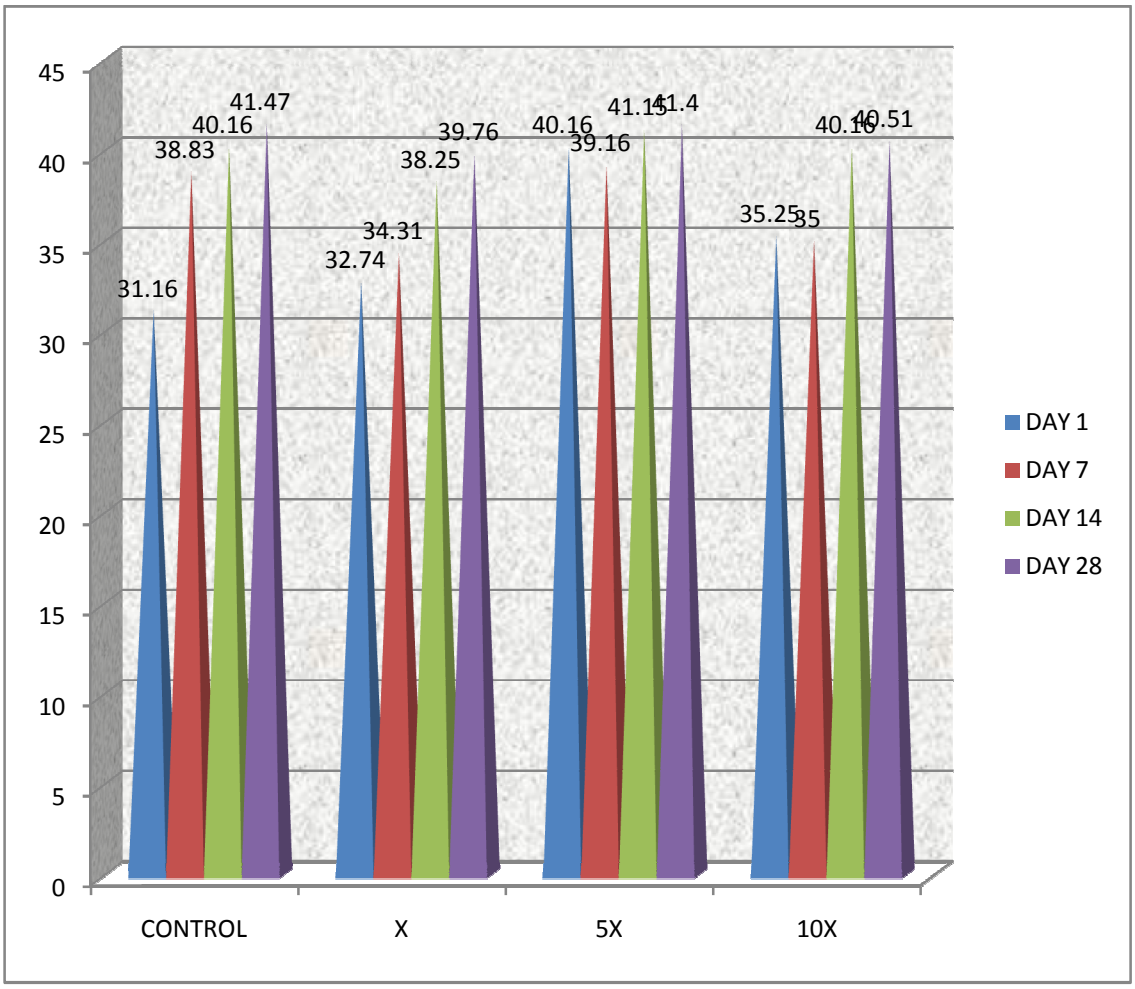
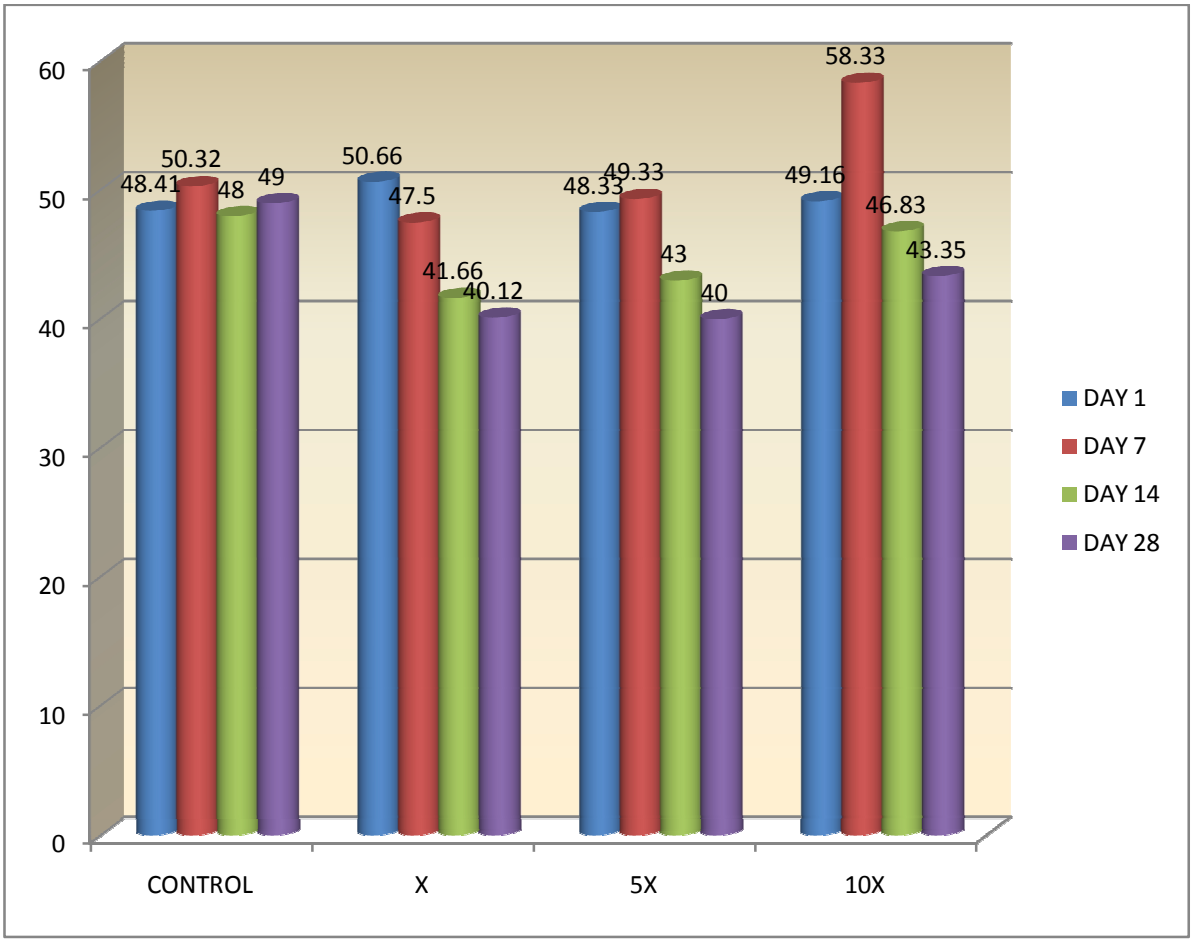


**CHART 3**

**THE AVERAGE WATER INTAKE BY CONTROL**

**AND TREATED GROUPS**



## CHART 4

## THE AVERAGE VALUE OF HAEMOGLOBIN, TOTAL WBC, PLATELETS AND RBC IN CONTROL AND TREATED GROUPS

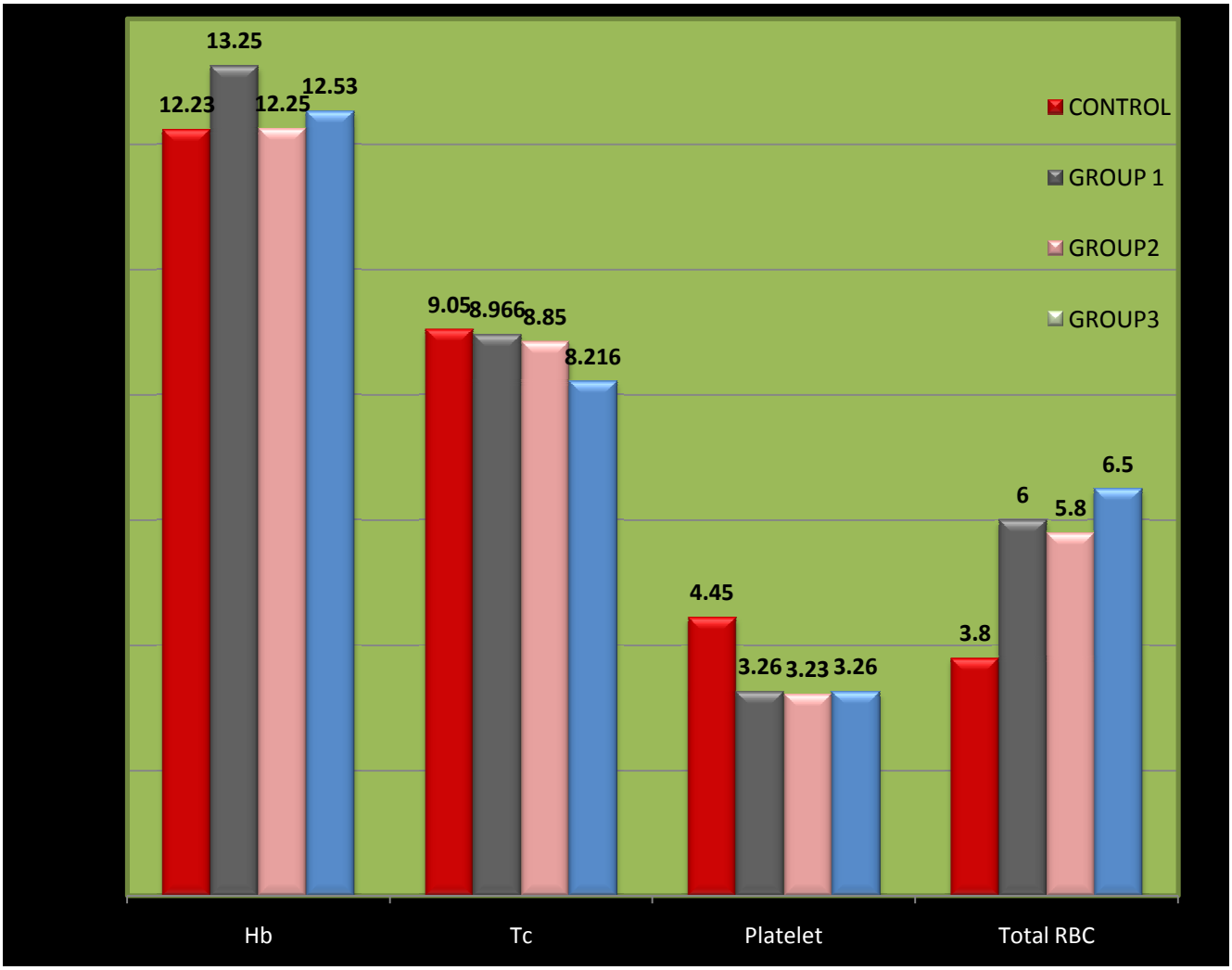
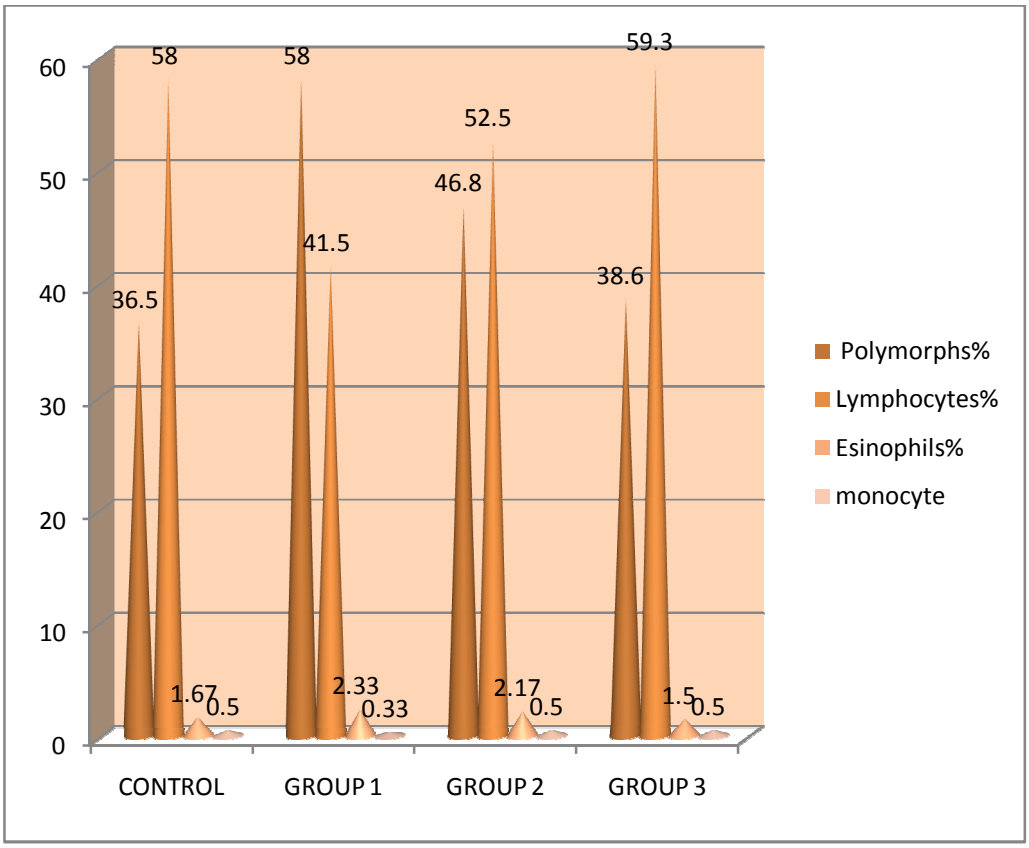


**CHART 5**

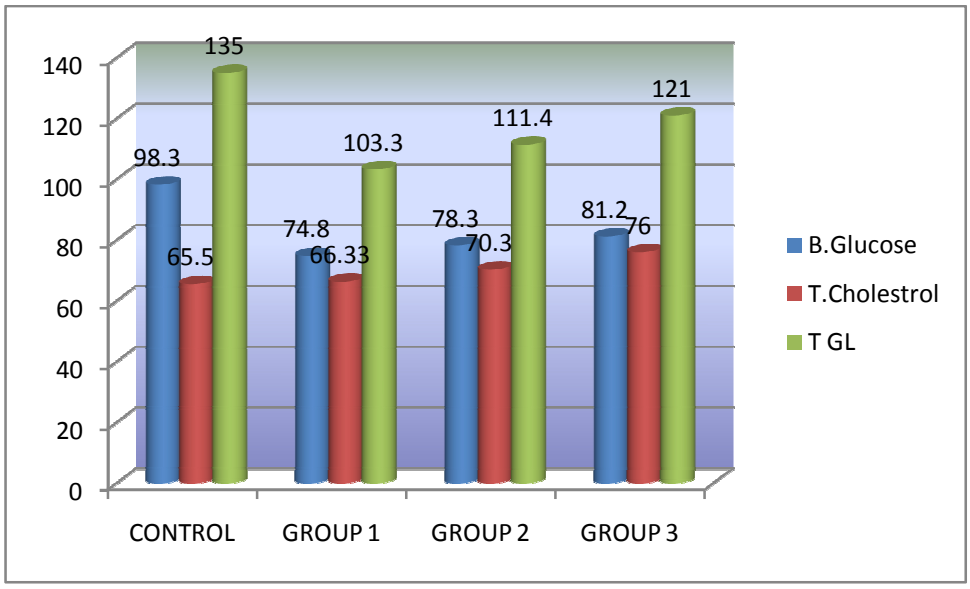
**THE MEAN VALUE OF DIFFERENTIAL COUNT OF**

**CONTROL AND TREATED GROUPS**



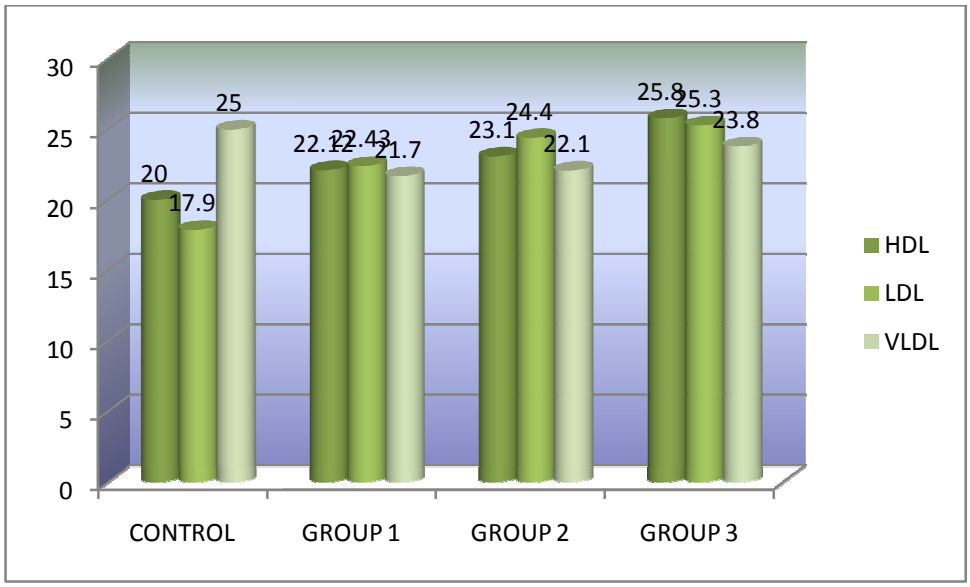
## CHART 6

## THE AVERAGE VALUE OF BOOD SUGAR, TOTAL CHOLESTEROL, AND TRIGLYCERIDES OF CONTROL AND TREATED GROUPS



## CHART 7

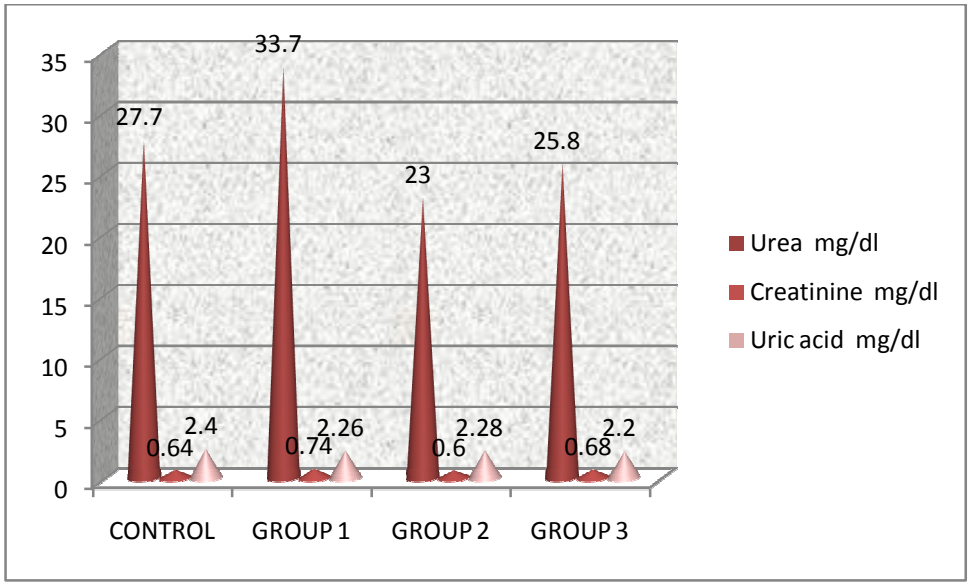
## THE AVERAGE VALUE OF HDL, LDL AND VLDL OF CONTROLAND TREATED GROUPS



## CHART 8

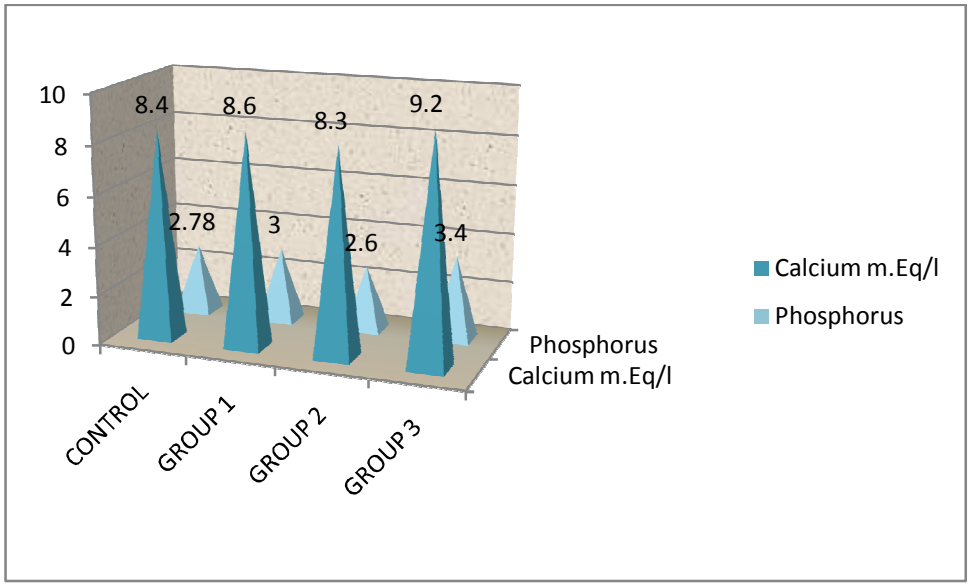
## THE MEAN VALUE OF UREA, URIC ACID, AND

## CREATINNE OF CONTROL AND TREATED GROUPS



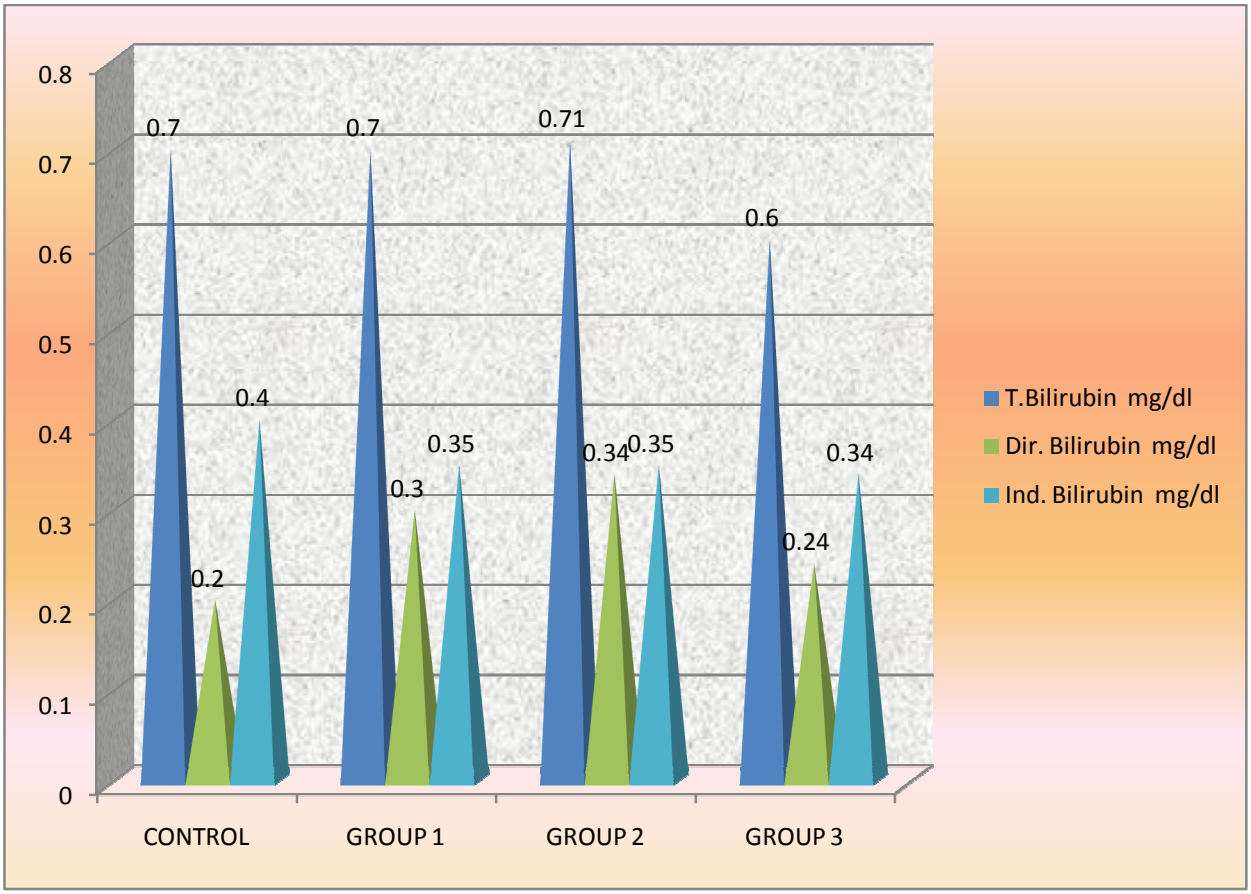
## CHART 9

## THE AVERAGE VALUE OF CALCIUM AND PHOSPHORUS OF CONTROL AND TREATED GROUPS



## CHART 10

## THE AVERAGE VALUE OF TOTAL, DIRECT, INDIRECT BILIRUBIN OF CONTROL AND TREATED GROUPS



## CHART 11

## THE MEAN VALUE OF TOTAL PROTEIN OF CONTROL AND TREATED GROUPS

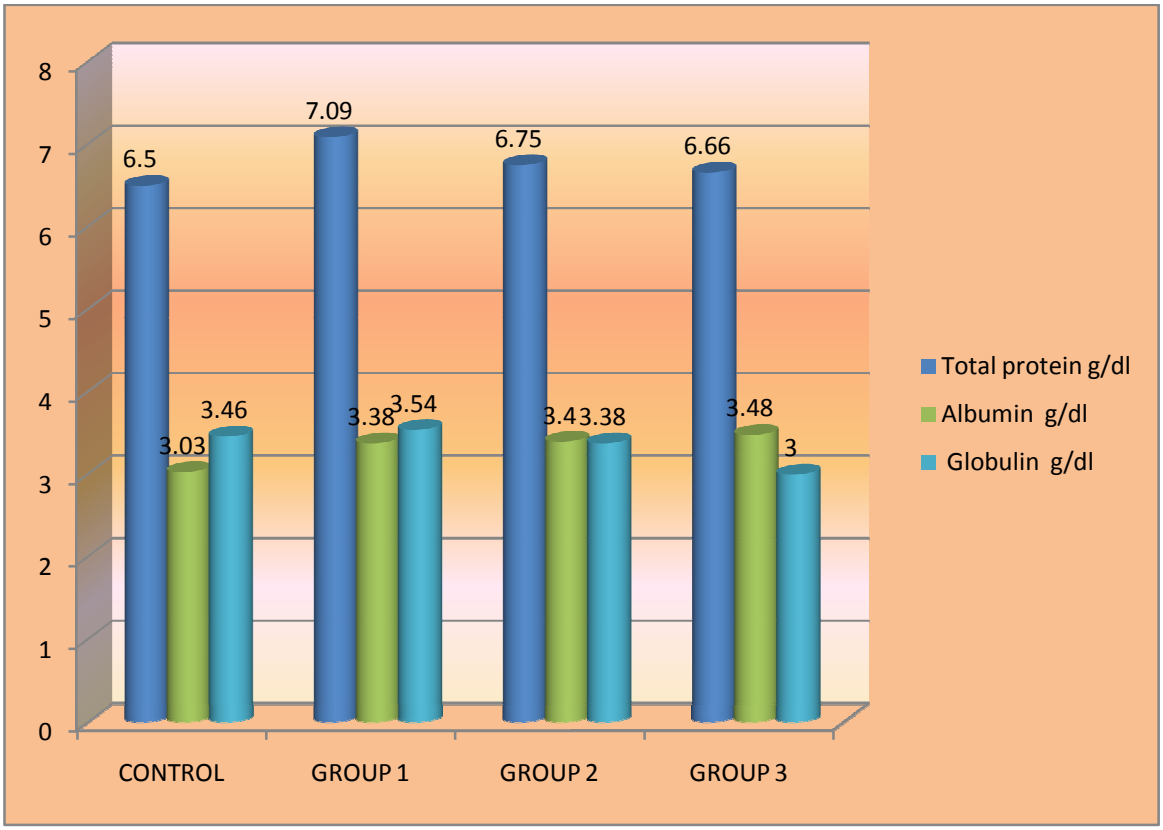
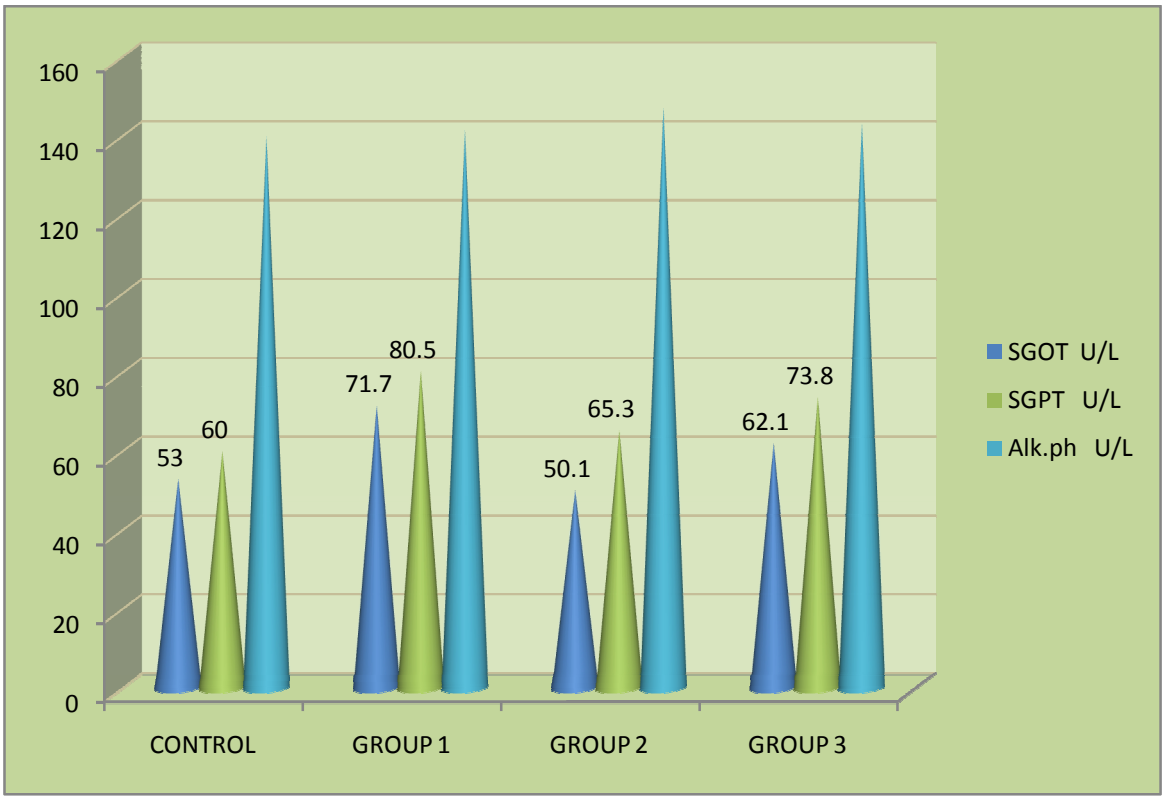


**CHART 12**

**THE AVERAGE VALUE OF SGOT,SGPT AND ALKALINE PHOSPHATASE OF**

**CONTROL AND TREATED GROUPS**



## 6.1 DISCUSSION

Qualitative, quantitative and toxicity studies of Seena rasa chendhuram were performed in this study. Qualitative analysis includes chemical analysis of unpurified and purified Gandhagam, unpurified and purified Padikaram and Seena rasa chendhuram. Quantitative analysis includes ICP-OES, HR-SEM, FTIR, XRF And finally both acute and repeated oral toxicity studies were carried out in Wister albino rats as per OECD guidelines.

The pH of the drug was 8.3 -8.5 (Table-4).It denotes weak alkalinity. Hence, in the oral administration of the drug it may absorbed quickly. Loss on drying of Seena rasa chendhuram at 105ºC is 7.83%(Table-4). This reveals that drug will not lose much of its volume on exposure to atmospheric air at room temperature. It shows that the drug has more stability.

Qualitative Analysis of unpurified and purified Gandhagam indicated the presence of iron, alkaloid and silicate. Qualitative Analysis of unpurified and purified Padikaram indicated the presence of iron, alkaloid and zinc. Analysis of the Seena rasa chendhuram showed the presence of lead, iron, alkaloid , sulphate and silicate. Although lead is present in the final preparation it comes under below detectable limit (BDL) in physiochemical analysis. It may be due to the different processes undergone by the ingredients during the purification and preparation of the test drug.

The **ICP-OES** results showed that As, Pb, Cd were found below detection level in of Seena rasa chendhuram. Since the heavy metal content in Seena rasa chendhuram was within normal and permissible limits, it may be safe for human consumption. (Table-6)

**HR SEM** analysis of Seena rasa chendhuram reveals the particle size as 25-75nm. The particles were homogenously distributed in the chendhuram. Smooth surface of the particles facilitate easy absorption in the gastro intestinal tract. Hence the drug will have increased bioavailability ((Table -3 and fig-9).

**FTIR** shows some specific functional groups like alcohols, carboxylic acids, esters, ethers in the chemical structure of Seena rasa chendhuram.

**XRF** results revealed that heavy metals like Hg, As, Cu, Fe were within permissible limits and the presence of important minerals like Mn, Ca, Cl and K.

In **Acute toxicity study** period there were no abnormal toxicity signs in the 5 and 50mg/kg body weight animals. There were reversible toxicity signs in 300mg/kg body weight animals. Two animals were found dead in 2000mg//kg body weight dose. There were no reduction in body weight of animals were observed in the study period. It concludes that **LD 50 cut-off** of Seena rasa chendhuram is **1000mg/kg body weight CAT-4(GHC)** as per the guideline OECD- 423.

**Repeated oral toxicity study** was conducted for about 28 days as per the OECD guideline-407 in 3 doses X(9.36), 5X(46.8), 10X(93.6).Animals were observed throughout the period. After 28 days animals were sacrificed and blood samples were collected, investigated and the results revealed that there were obvious changes in Hb and mild changes in other hematological parameters compared to control group. Finally all the reports were statistically calculated. There was no significant change in body weight, water and food intake, hematological and bio chemical parameters.

The **histopathological study** on the organs such as heart, lungs, kidney, spleen, liver and Stomach was normal in control, low dose, and mid dose groups. In high dose group in liver portal tract shows lymphocyte infilteration.

# SUMMARY

***Seena Rasa Chenduram*** was taken as dissertation drug for the evaluation of its toxicity profile. The drug was prepared from mercury ***(Rasam),*** sulphur***(Gandhakam)*** and alum***( padikaram)*** .The drug was chosen from the siddha literature Anuboga vaidhya navaneetham.The above ingredients are used to cure chronic, infectious, non infectious ailments in Siddha system of Medicine. The raw drugs were procured from country drug shop and authenticated at Siddha Central Research Institute,chennai.The ingredients were purified and the medicine was prepared as mentioned in the Siddha literature.

Seena rasa chendhuram was analysed qualitatively with physico chemical and chemical analysis. Quantitatively with ICP-OES, FTIR, XRF and HR-SEM analysis. Acute and repeated oral toxicity were conducted as per the OECD guidelines.

Initially the test drug was subjected to **physicochemical analysis.** It has given the pH and purity of the drug. Then the samples were analysed for chemical constituents. It reveals that the presence of important constituents.

The **ICP-OES** results showed the heavy metals like As, Pb, Cd were found below detection limit in Seena rasa chendhuram.

**HR SEM** analysis of Seena rasa chendhuram reveals the particle size as 25-75nm. The particles were homogenously distributed in the chendhuram.

**FTIR** shows some specific functional groups like in the chemical structure of Seena rasa chendhuram.

**XRF** results revealed that heavy metals like Hg, As, Cu, Fe were within permissible limits and the presence of important minerals like Mn, Ca, Cl and K.

The Acute toxicity study results revealed that no mortality in 5,50,300mg/kg body weight group animals. Two animals were found dead in 2000mg//kg body weight dose.It concludes that **LD 50 cut-off** of Seena rasa chendhuram is **1000mg//kg body weight** as per the guideline OECD- 423 and comes under **CAT-4(GHC).**

**Repeated oral toxicity study** was conducted for about 28 days as per the OECD guideline-407 in 3 doses X(9.36), 5X(46.8), 10X(93.6).Animals were observed throughout the period. There were no abnormal toxic signs were found during the study period. There were no remarkable weight reduction in animals.

Although the human dose was prescribed for 5 days , the period of 28 days administration did not produced any mortality in repeated oral toxicity study.

# 8. CONCLUSION

As the result of this study, it has been concluded that,

The Acute and Repeated oral toxicity study of Seena rasa chendhuram is found to be less toxic and the therapeutic dose level mentioned in the literature is safe for human consumption.

As qualitative and quantitative analysis reveals that the presence of heavy metals within permissible limits, this in turn reveals the potential knowledge of Siddhars in the field of metals and minerals in medicine preparations.

Hence further studies on Seena rasa chendhuram are to be conducted for its scientific validation and global acceptance.

## CHAPTER IX

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*This Certificate is awarded to ~~Mr~~/~~Ms~~/Dr.....S. SUDHA.....*

*for participating as a ~~Resource Person~~ / Delegate in the VII Workshop*

*on* **"Research Methodology & Biostatistics"**

*for AYUSH Post-Graduates & Researchers*

*organized by the Department of Siddha*

*The Tamil Nadu Dr. M.G.R. Medical University*

*from 6th Feb. 2012 to 10th Feb. 2012.*

**DR. MAYILVAHANAN NATARAJAN**
M.S.Orth. M.Ch.Orth. (L'pool) Ph.D. (Orth. Onco.) F.R.C.S. (Eng) D.Sc.
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SOPHISTICATED ANALYTICAL INSTRUMENT FACILITY
INDIAN INSTITUTE OF TECHNOLOGY, MADRAS
Chennai - 600 036. INDIA

# CERTIFICATE

Certified that herbo-mineral drug **SEENA RASA CHENDURAM** formulated by **Dr.S.Sudha** III Year M.D(S) Department of Nanjunool ,National Institute of Siddha , Tambaram Sanatorium was analysed (quantitative) by ICP-OES, FT-IR, HR-SEM and Physico chemical Analysis Methods at SAIF, IITM, Chennai-36, during December 2012.

Dr. R. MURUGESAN
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IAEC PROTOCOL NO: 1248/ac/09/CPCSEA/4-39/2011

20/12/2011

**CERTIFICATE**

**This is certify that the project title...** A toxicity studies on ..... "SEENARASA CHENDHURAM" ..... **has been approved by the IAEC.**

**Name of Chairman/Member Secretary IAEC:** Dr. K. MANICKAVASAKAM

**Name of CPCSEA nominee:** Dr. B. JAYACHANDRAN DAVE

**Signature with date**

**Chairman/Member Secretary of IAEC:**

**CPCSEA nominee:**

**(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)**

சித்த மருத்துவ மைய ஆராய்ச்சி நிலையம், அரும்பாக்கம், சென்னை - 600 106
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Ministry of Health & Family Welfare, Govt. of India)
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Web: www.crsiddha.tn.nic.in

08.10.2012

## CERTIFICATE

Certified that the minerals submitted for identification by Dr. S. Sudha, III year M.D., Department of Nanju Noolum Maruthuva Neethi Noolum, National Institute of Siddha, Tambaram Sanatorium, Chennai-47 are identified as Rasam – Mercury, Ganthagam – Sulphur, Padikaram – Aluminium Potassium Sulphate.

(R.Shakila )
Research Officer (Chemistry)

(S. Jega Jothi Pandian)
Asst. Director- In charge